Synthesis of New Benzimidazole-derived Epothilone Analogues

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List of Abbreviations

Ar	Aromatic
Ac	Acetyl
AcOH	Acetic acid
Bn	Benzyl
B(OMe)$_3$	Trimethyl Borate
Boc	tert-Butyloxycarbonyl
BP	Boiling point
C-(1-19)	Carbon at position (1-19) of Epothilone Ring
°C	Centigrade
CH_3CN	Acetonitrile
CH_2Cl_2	Dichloromethane
DMAP	4-(Dimethylamino)-pyridin
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EtOH	Ethanol
Epo	Epothilone
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
E	Entgegen (Opposite)
HF	Hydrogenfluoride
HYTRA	2-Hydroxy-1,2,2-triphenylethyl acetate
HPLC	High Performance Liquid Chromatography
hr	Hour
IBCF	Isobutyl chloroformate
IC50	Inhibit Cellular Proliferation by 50%
IR	Infrared
lk	Like
MeI	Methyl Iodide
Min	Minutes
mmol	Millimole
mL	Milliliter
MS	Mass Spectrometry

MTAs	Microtubules Targetting Agents
MCF-7	Michigan Cancer Foundation - 7
MP	Melting point
PDC	Pyridinium Dichromate
Ph	Phenyl
i-Pr	Isopropyl
Py	Pyridine
RCM	Ring Closing Metathesis
R	Rectus (right-handed)
$\mathbf{R}_f$	Retention Factor
S	Sinister (left-handed)
LAH	Lithium Aluminium Hydride
LDA	Lithium Diisopropyl Amide
TBDMS	tert-Butyldimethylsilyl
TMP	Trimethoxyphenyl
TBSOTf	tert-Butyldimethylsilyl trifluoromethanesulphonate
TESOTf	Triethylsilyl trifluoromethanesulfonate
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
THF	Tetrahydrofuran
TPAP	Tetra-n-propylammonium perruthenate
$\mathbf{NEt}_3$	Triethylamine
$\mathbf{MgSO}_4$	Magnesium Sulfate
$\mathbf{NH}_3$	Ammonia
NCI	National Cancer Institute
NMO	N-methylmorpholine
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
SAR	Structure Activity Relationship
UV	Ultraviolet
Z	Zusammen (Together)

Abstract

The total synthesis of a new benzimidazol-derived epothilone analogue was carried out in this work by using the robust strategy developed by Schinzer group. This analogue of epothilone does not exist in nature, hence, it is synthesized chemically in order to find a more biologically active molecule. For this purpose, a novel strategy is used for the synthesis of building block **126**. The required diastereomeric configuration of building block **106a** is achieved by using stereoselective aldol reaction between building blocks **93** and **65**. At the end, ring closing metathesis for diene **130** is used for the synthesis of epothilone ring **136**.

Die robuste Synthese eines neuen Benzimidazoled-basierten Epothilon- Analogons wurde in der Dissertation durchgeführt. Dieses Epothilon- Analogon kommt nicht in der Natur vor. Es wurde chemisch synthetisiert, um ein biologisch aktiveres Derivat zu finden. Die Konfiguration des dafür notwendigen Bausteins 126 wurde dabei durch eine vorausgehende stereoselektive Aldol Reaktion aus **93** and **65** erzeugt. Das Makrolid wurde durch eine Ringschluß Olefin-Metathese erzeugt und führte dann zu dem neuen Epothilon Analogon **136**.

Statement of Autonomy

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by any other person, nor any material which has been substantially accepted for the award of any other degree or diploma at the University of Magdeburg or any other educational institution, except where this is appropriately mentioned in the thesis. Any contribution to research made by other persons with whom I have worked at the University of Magdeburg or elsewhere has been explicitly acknowledged in this thesis.

I also declare that the intellectual content of this thesis is the product of my own work, except where the assistance of others in the design and conception of the project or in style, presentation and linguistic expression is acknowledged.

Magdeburg, 02.03.2022
Muhammad Zahid Iqbal

Contents

Chapter 1

Introduction

After heart diseases, cancer is the most common cause of death in humans. Basically, cancer is a generic term that refers to its two main characteristics, namely uncontrolled cell growth and the ability to spread to other parts of the body (metastasis). Because of metastasis, it is difficult to treat cancer with surgical means. Under these circumstances, chemotherapy offers an excellent method of treatment. Natural products have made an enormous contribution to cancer chemotherapy, and more than half of the anti-cancer drugs currently in clinical use are natural products or derived from natural products.[1]

The ultimate goal of cancer chemotherapy is to develop a drug that specifically destroys the malignant cells and differentiates between them and normal cells. Cancer can actually be defined as an overproduction of cells when the delicate balance between cell growth and cell death is disturbed. In order for cell division to take place through mitosis, microtubules play a very important role in the separation of chromosomes through spindle formation. In principle, mitosis can therefore be controlled by influencing the polymerization of the microtubules.

In general, microtubules are one of the main components of the cytoskeleton, which are essential for many cellular processes such as the maintenance of cell structure, protein transport and mitosis. Microtubules are also known as conveyor belts inside the cell.[2] Mitosis in cancer cells can therefore be controlled or stopped by degrading the microtubules, as a result of which the cancer cells no longer multiply and the tumor consequently no longer grows.[3]

1.1 Structure Of Microtubules

The Microtubules are composed of dimers of the protein tubulin. The heterodimer consists of α-tubulin and β-tubulin (with a size of about 40Å). The

two subunits are homologous. These dimers are arranged head-to-tail in rows called protofilaments. A varying number of protofilaments (between 11 and 17) form the hollow tube that constitutes the microtubule. In vivo, most microtubules contain thirteen protofilaments. Each protofilament is staggered about 9Å with respect to its neighbors, so that the tubulin subunits describe helices. The diameter of microtubules is about 240Å.[4]

Each dimer in the protofilament has a specific direction, as the α-tubulin and β-tubulin are not identical. The protofilaments arrange themselves in such a way that the directionality is retained in the microtubule. Therefore, one end of the microtubule is called the $(+)$ end and the other end is called the $(-)$ end. In a free microtubule, the tubulin dimers keep adding at the plus end and keep falling off at the minus end. This is called treadmilling.[5]

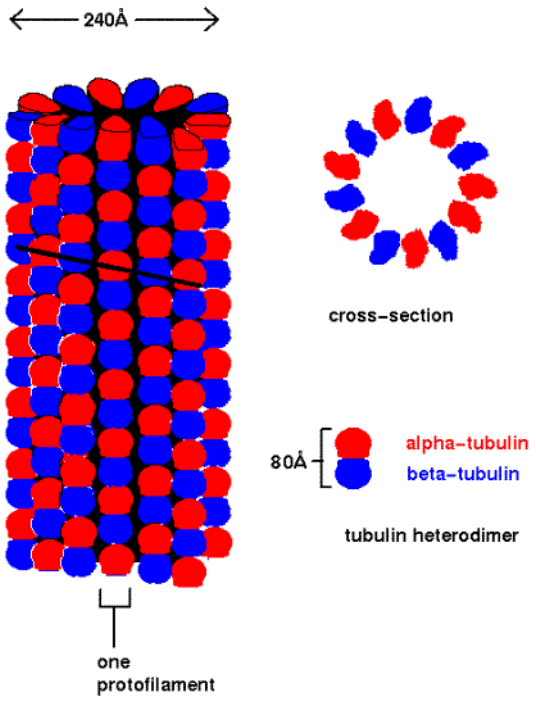

Figure 1.1: Structure of a singlet microtubule, indicating slant

Thus, the active substance that interferes with the dynamics of microtubule stability can be clinically referred to as an anticancer or antimicrotubule agent (microtubule-targeting agent).

1.2 Microtubules Targeting Agents

The microtubules targeting agents (MTAs) are the natural products derived from bacteria, marine sponges, molluscs and plants. Depending on their mode of action on microtubule polymers, MTAs can be classified as either microtubule stabilizing agents or microtubule destabilizing agents.

1.2.1 Microtubules Destabilizing Agents

The microtubules-destabilizing agents, according to their binding sites to receptors, can be classified into two main classes such as colchicine and vinca alkaloids locations. The binding location of the colchicine and vinca alkaloids was uncertain for a long period of time but finally it was discovered that these two compounds target microtubules at different positions.

Colchicine

Colchicine was first antimitotic destabilizing agent discovered from plant extracts. It consists of three rings, trimethoxyphenyl (TMP, ring A), a methoxytropone (ring C) and a seven-membered ring (ring B) bearing an acetamido substituent at its C7 position.

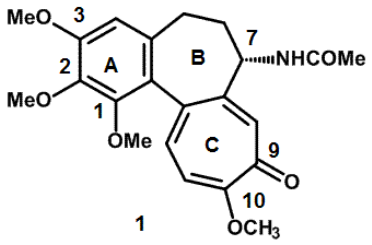

Figure 1.2: Structure of colchicine

Due to its high toxicity, colchicine site inhibitors have yet not been able to get any clinical significance.[6]

Vinca Alkaloids

Vinca alkaloids are an extract of compounds that are often derived from plants. The main mechanisms of vinca alkaloids cytotoxicity is due to their interactions with tubulin and disruption of microtubule function, specifically targeting the microtubules that form the mitotic spindle apparatus, leading directly to metaphase

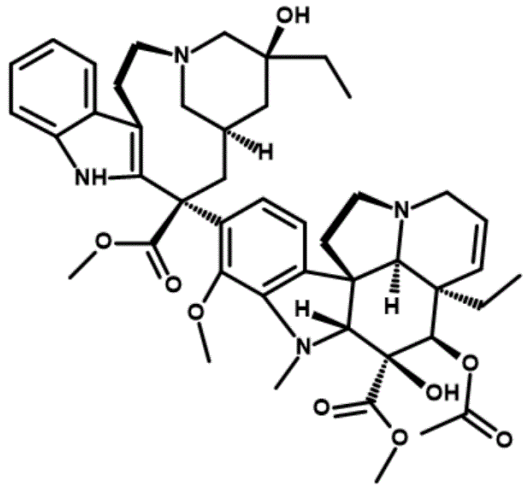

Figure 1.3: Structure of vinblastine

arrest. The vinca alkaloids bind to binding sites on tubulin that differ from those of the taxanes and colchicine. The vinca alkaloids are generally used in combination with other cancer drugs for chemotherapy in patients.

1.2.2 Microtubules Stabilizing Agents

The microtubules-stabilizing agents can be divided into two main classes such as taxanes and epothilones, both which bind to the same domain of β-tubulin of heterodimers.

Taxanes

Paclitaxel was isolated at the American National Cancer Institute (NCI) in 1962. Paclitaxel was widely ignored as an antimitotic agent because it was hardly available and poorly soluble.

However, when its mechanism of action as microtubule stablizer rather than destabilizer was discovered, interest in researching it for medical use increased dramatically. Paclitaxel is the most important chemotherapeutic agent for the treatment of ovarian, breast and prostate cancer. It was commercially developed by Bristol-Myers-Squibb (BMS) under the generic name of paclitaxel and is marketed under the trade name of Taxol®. In the presence of paclitaxel, the microtubule cytoskeleton is reorganized and extensive parallel arrays or stable bundles of microtubules are formed in cells growing in tissue culture.[8]

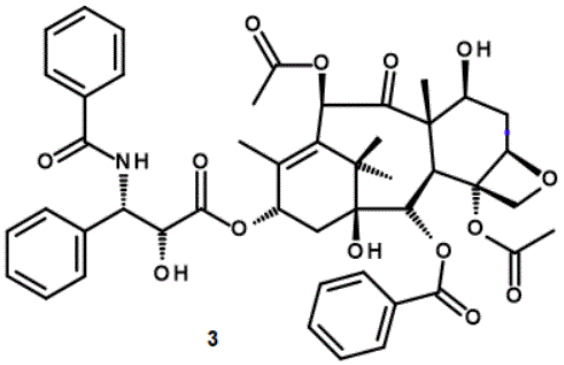

Figure 1.4: Structure of paclitaxel

The success of paclitaxel led to the development of many of its analogues. Docetaxel was discovered as a late intermediate in a synthetic strategy for paclitaxel and showed a similar binding effect to paclitaxel, but with a fourfold higher potency and better water solubility. This improved availability combined with the increased potency, broad spectrum of clinical activity and reduced side effects provided a strong foundation for the future success of Taxotere as a clinically useful drug.[9]

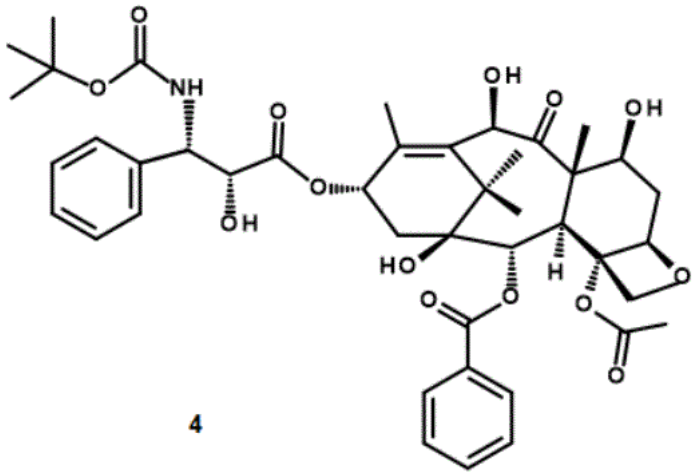

Figure 1.5: Structure of docetaxel

Eleutherobin

Eleutherobin is a novel natural product isolated from a marine soft coral. It is highly effective in inducing tubulin polymerization in vitro and is cytotoxic to cancer cells with an IC_{50} like that of paclitaxel. This compound, along with other multidrug-resistant agents, is cross-resistant to P-glycoprotein-expressing cells and is cross-resistant with paclitaxel to a cell line that has altered tubulin.

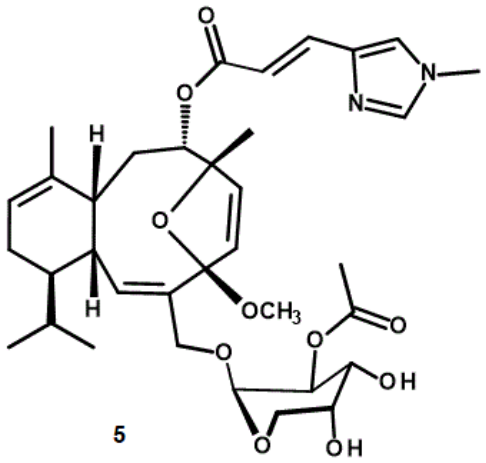

Figure 1.6: Structure of eleutherobin

In mechanistic studies, eleutherobin shares with paclitaxel the ability to induce tubulin polymerization *in vitro* and is most likely cytotoxic by virtue of this mechanism. Human colon carcinoma cells exposed to eleutherobin contain multiple micronuclei and microtubule bundles, and they arrest in mitosis, depending on concentration, cell line, and length of exposure. These morphological abnormalities appearing in cultured cells are indistinguishable from those induced by paclitaxel. Electron microscopy reveals that eleutherobin induces homogeneous populations of long, rigid microtubules similar to those formed by paclitaxel. Thus, eleutherobin is a new chemotype with a mechanism of action like that of paclitaxel and, as such, has promising potential as a new anticancer agent.[10]

Discodermolide

(+)-Discodermolide, a polyhydroxylated lactone isolated from the marine sponge *Discodermia dissoluta*, is a promising antitumor agent that continues to be the subject of intensive chemical, biological, and pharmaceutical research since its discovery two decades ago. Although structurally different from Taxol®,

discodermolide shares a common mechanism of action as a potent tubulin polymerizer that leads to blockade of cells in the G2/M phase of the cell cycle, impairment of microtubule function and cell death.

After Novartis AG received a license for an antitumor agent from the Harbor Branch Oceanographic Institution in 1998, discodermolide was given unprecedented priority for synthesis on an industrial scale, in some cases adopting and modifying academic synthesis schemes to produce sufficient quantities for clinical trials.[11]

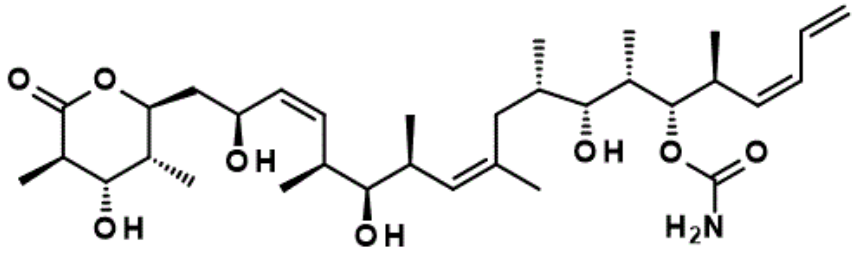

Figure 1.7: Structure of discodermolide

Epothilone

Epothilone occupies a very important place in this class of natural products due to its better properties in the fight against cancer and its better solubility in water. The discovery of epothilone, its properties, structure-activity relationship and synthesis strategies are discussed in the following chapters of this thesis.

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Chapter 2

Epothilone

The discovery of epothilone was the result of basic research with the aim of identifying new producers of diverse secondary metabolites among bacteria. It was found that myxobacteria *sorangium cellulosum* synthesized some compounds first isolated in 1987 by Reichenbach and Hoefle.[12]

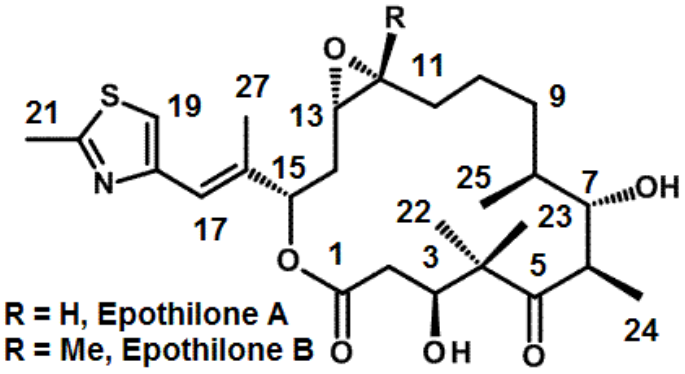

Figure 2.1: Structure of epothilone A and B

In 1993, Taxol® was developed as new antitumor agent with a new mechanism of action that inhibits cell division by stabilizing of microtubules. Taxol® was approved for the treatment of ovarian cancer but showed undesirable side effects. The researchers were therefore looking for a more suitable and better alternative. In 1995, Bollag identified these compounds as being microtubule-stabilizing agents with a Taxol® like mechanism of action, most of which had diverse and novel structures and unusual and interesting mechanisms of actions against cancer cells.[13]

At that time, the name epothilone was coined from the structural features epoxide, thiazole and ketone (see structure in **Figure 2.1**). Epothilone was found to have several properties that could make it superior to Taxol®. For example, epothilone required low dose and showed activity against multidrug-resistant cells and also against Taxol® resistant cancer cells. In contrast to Taxol®, it is more soluble in water. In addition, epothilone can easily be obtained either by total synthesis or by fermentation. So these compounds epothilone A and specially B appeared to be even more active than Taxol® in the tubulin polymerization assays.[14]

A comparison of activities of epothilone B with the clinically accepted anticancer drug Taxol® provides excellent information for the evaluation of their potential as anticancer agents (see **Table 2.1**). Epothilone B in comparison with Taxol® proved to be highly active against human cancer cell lines.[14]

				Average tumor volume (T/C)			
Tumor	Drug	Route	mg/Kg	Day10	Day15	Day20	Day25
CCRF-CEM	Control	i.p	0.0	1.0	1.0	1.0	1.0
		i.p	1.5	0.4	0.4	0.37	0.34
	Epo B	Water	3.0	0.41	0.35	0.38	0.50
		i.v	1.5	0.38	0.34	0.42	0.37
	Epo B	DMSO	3.0	0.28	0.38	0.29	0.24
		i.p	20.0	0.33	0.28	0.31	0.34
	Paclitexal	DMSO	30.0	0.43	0.25	0.23	0.25

Table 2.1: Activity of epothilone and paclitaxel *in Vivo* Data

These values indicate that epothilone B is even more active against cancer cells than Taxol®.[15]

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2.1 Physical Properties of Epothilone

Epothilone A and B can be obtained as white crystalline solids with melting points from 76 °C to 128 °C depending upon the crystal form. Epothilone A is well soluble in organic solvents such as methanol, ethyl acetate, acetone, diethylether and dimethyl sulfoxide. While epothilone B is comparatively less soluble,[16],[14] both epothilone A and B are sparingly soluble in benzene, petroleum ether and toluene. The aqueous solubility of epothilone A is 722 mg per liter of water[17] and that of epothilone D is 24 mg per liter water.[13]

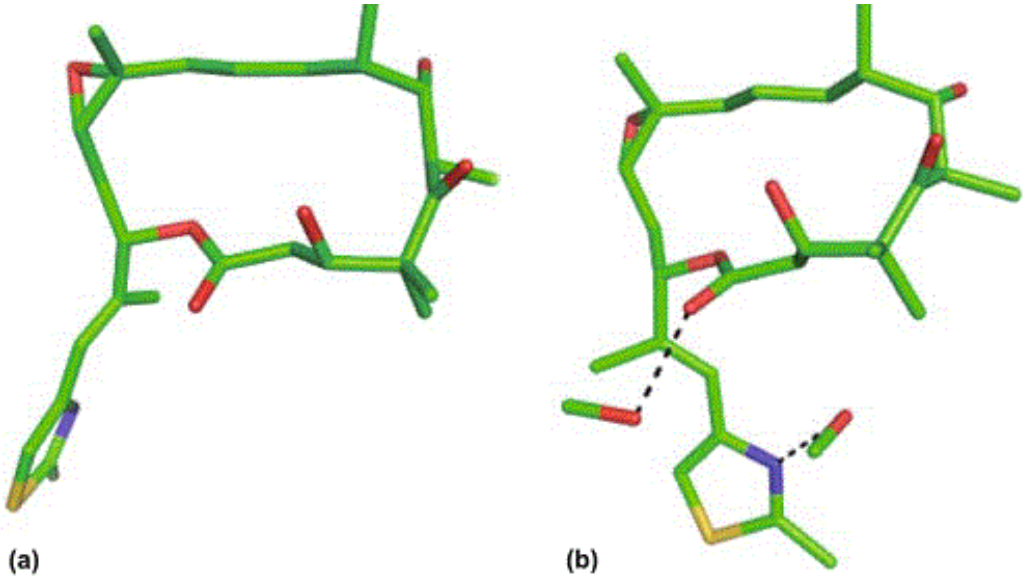

Figure 2.2: Crystalline structure of epothilone

X-ray images of the epothilone acquired by using the diffraction pattern (see **Figure 2.2**) show that the thiazole side chain can adopt different conformational forms. Interestingly, the solvation system used for the crystallization of epothilone B is a crucial factor that determines the conformational forms of its thiazole ring. The X-ray image of epothilone B in **Figure 2.2(a)** was obtained from crystals by using solvation system consisting of dichloromethane and petroleum ether. While the X-ray image in **Figure 2.2(b)** was obtained by using methanol and water as the solvents for crystallization.[16]

2.2 Structure Activity Relationship

With the help of synthetic analogues, it was possible to derive a model for understanding the relationship of structural properties and activity against cancer cells. Natural epothilone is a 16-membered lactone with thiazoloylethylene side chain both spiked with methyl and oxygen groups in position of a polyketide. Epothilone can be divided into four parts called as A, B, C and D as shown in **Figure 2.3** to understand the effects of the structural change in the earlier mentioned regions of epothilone on its activity against cancer cells.

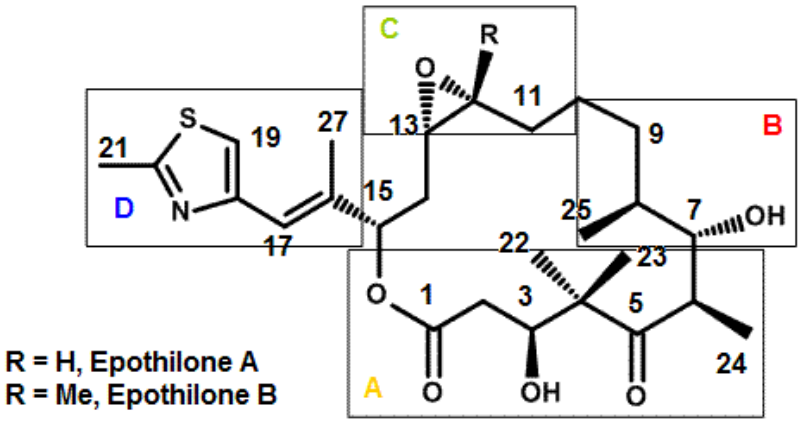

Figure 2.3: Regions of epothilone for microtubuline binding

It is generally recognized that a 16-membered macrocycle and the stereochemical assignment of regions A,B,C and D are important prerequisites for the biological activity of epothilone. However, good to moderate activity is observed by certain 17 and 18-membered macrocyclic epothilones.[18]

2.3 Modifications of Region A

The modification of the hydroxyl group at C-3 bond of natural epothilone is investigated by replacing it with cyano group, as shown in structure **7** of **Figure 2.4**. However, only twofold lower activity was observed for structure **7** compared to epothilone A.[19]

While modification between C-2$-$C-3 of natural epothilone by having an α-β unsaturation as shown in structure **8** of **Figure 2.4** in reference to epothilone B showed fourfold lower activity against the human colon carcinoma cell line.[20]

While saturation of the double bond and removal OH group at C-3 in structure **9** of **Figure 2.4** showed comparable activity against human cervix carcinoma cell lines.[20] These findings established the fact that the 3-hydroxyl group in epothilones even in the absence of a strong conformational constraint at C-2−C-3 bond is not an important prerequisite for potent biological activity against cancer cells. However, it is important to note that a significantly reduced tubulin polymerizing activity is reported by inversion of stereochemistry at C-3 in epothilone C.[21]

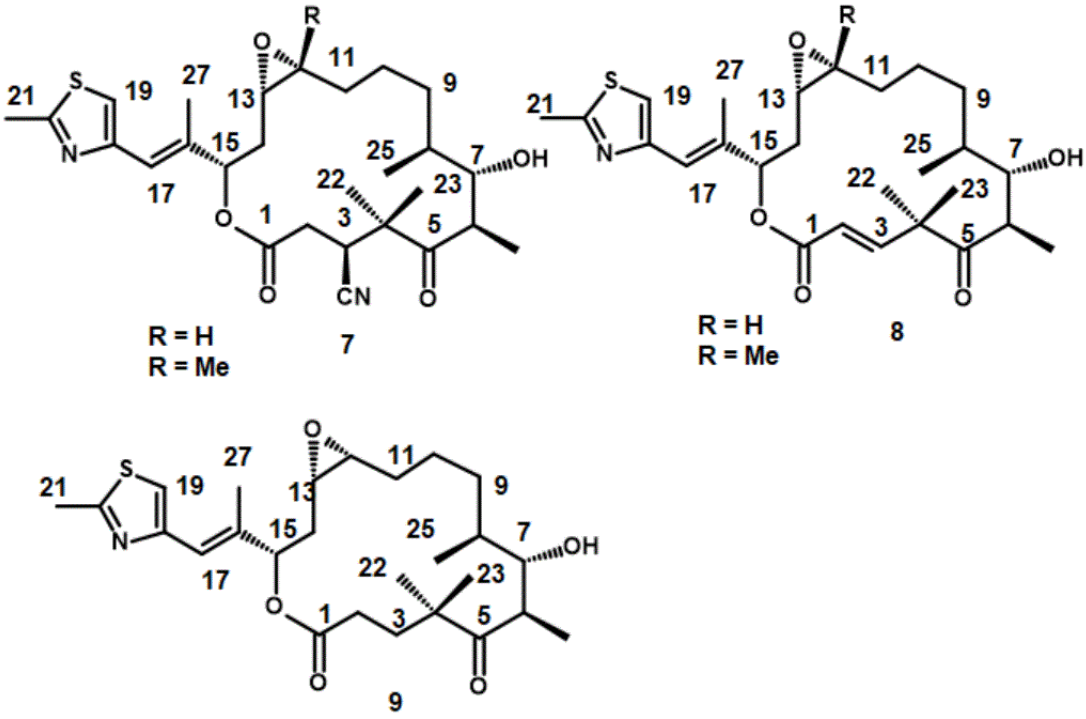

Figure 2.4: Modification of epothilone at C-3 position

Furthermore, modification of C-4 has been reported by Nicolaou *et al*. to study the effects of modifications on the activity of an analog with a 3-membered ring at C-4 (structure **10** of **Figure 2.5**) and by changing the stereochemical positions of methyl and hydrogen (structure **11** of **Figure 2.5**).[21] Interestingly, C-4-mono methylated epothilone A derivatives have been reported to exhibit similar growth inhibitory activity against the mouse fibroblast cell line L929 compared to epothilone A. Thus, contrary to previous assumptions, it is proved that *gem*-dimethyl at C-4 position does not play a crucial role in stabilizing of bioactive conformation of epothilone. Furthermore, modification at C-5 position showed that ketone at this position is very important for biological activity against cancer cells.[22],[23]

So in short, modification effects can be summarized on the structure activity relationship of this part of epothilone in such a way that C-3 stereochemistry is important, but C-2−C-3 olefin is tolerable and C-4 *gem* dimethyl can be replaced by a cyclopropyl while the ketone at C-5 position is very important for the activity of epothilone against cancer cells.[23]

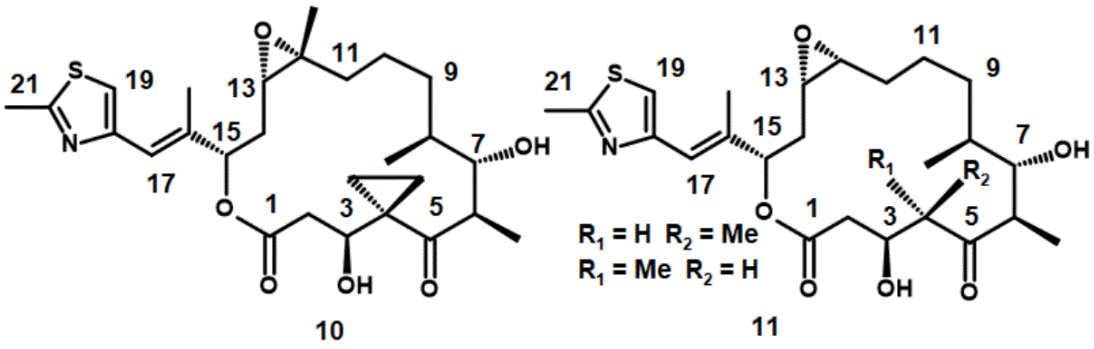

Figure 2.5: Modifications of epothilone at C-4 position

2.4 Modifications of Region B

The C-6-desmethyl and further modification at C-6 epothilone (synthesized by Schinzer group) have been extensively studied by the group at Schering AG (now Bayer). Since, relatively little biological data is known for this class of analogues, it is clear that the extension of C-6 methyl to ethyl increases the activity fourfold compared to epothilone B, while allyl and propyl derivatives are only slightly less active than the parent compound (see **Figure 2.6**).[24]

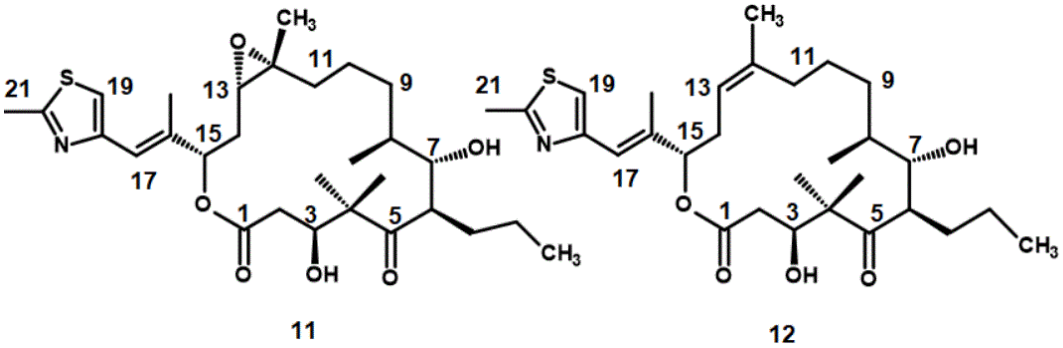

Figure 2.6: Modifications of epothilones at C-6

Similarly, the C-6-propyl analogues of epothilones B and D (**Figure 2.6**) were found to have IC_{50} values of 3.4 nM and 38 nM, against the human breast cancer MCF7 cell line compared to their parent compounds epothilones B and D, which had 0.6 nM and 19 nM respectively. Interestingly, these compounds also retain their full activity against the multi-drug-resistant NCI/Adr variant of the MCF7 line.[25]

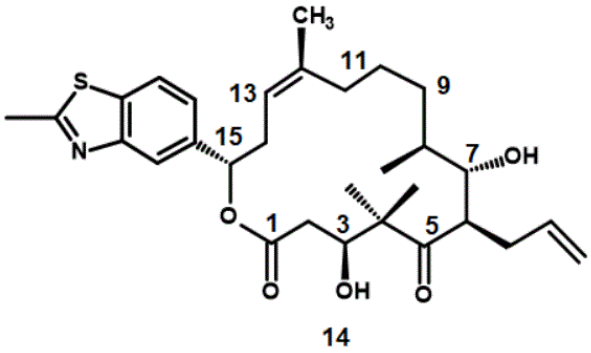

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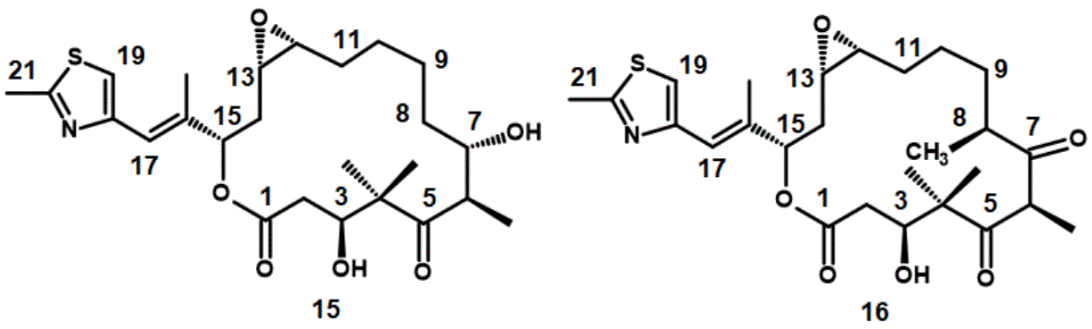
Figure 2.7: Structure of Schering ZK-Epo (Sagopilone)

Most interestingly, the derivative of epothilone B [(ZK-Epo (Sagopilone)] with 6-allyl at C-6 and a further modification in heterocyclic ring developed by the group at Schering was tested in Phase II clinical trials, but later the trials (Phase III) were discontinued by Bayer management.[26] It was well known that changes at C-7 and C-8 (see **Figure 2.8**) generally lead to a general loss of activity against cancer cells. Thus, removal of the methyl group at C-8 position resulted in a 100-fold loss of activity against cancer cells as compared to epothilone A (structure **15** of **Figure 2.8**).[27]



15 16

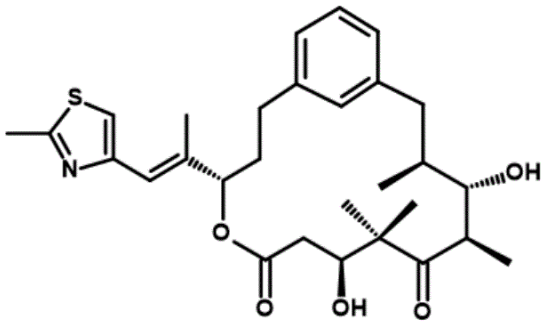
Figure 2.8: Modification of epothilone at C-7 and C-8

Similarly in the literature, inversion of stereochemistry at C-8 led to a loss of

activity.[21] However, the literature does not report how inversion of stereochemistry at C-7 position would affect the activity against cancer cells. But having, a ketone at C-7 position, as shown in structure **16** in **Figure 2.8** resulted in a significant loss of activity.[28]

2.5 Modifications of Region C

The modifications of region C reported by Nicolaou and Danishefsky of natural epothilone referred in **Figure 2.3** as region C, the C-9 to C-11 trimethylene segment adjacent to the epoxide, by removal or addition of methylene in this region generally resulted in a loss of activity against cancer cells.[18] The Novartis group took a new approach based on molecular modeling. They decided to incorporate a phenyl ring into C-9 to C-12 region of epothilone, as shown in structure **17** of **Figure 2.9**. Unfortunately, this molecule compared to epothilone B showed less activity against cancer cells.[29]



17

Figure 2.9: Modification of epothilone at C-9 and C-12

Despite of some earlier disappointments, structural variations in the C-9 to C-11 trimethylene segment yielded a number of highly potent epothilone analogues and some of them exhibited very favourable pharmacological properties *in vivo*. The identification of a series of natural epothilones with an olefin at the C-10−C-11 position and the development of more reactive metathesis catalysts provided an opportunity to reinvestigate the applicability of this reaction to close the macrocyclic ring of epothilone.

Thus, the structures **18-21** were synthesized by ring-closing metathesis approach to generate double bonds in epothilone ring. The epothilone structure **18a**, shown in **Figure 2.10**, showed three- to fourfold lower activity against

MCF7 breast, SF268 glioma, NCIH460 lung cancer and HL60 promyelocytic leukemia cell lines compared to epothilone D, while compounds **18-21** were equipotent against the human T-cell leukemia cell lines CCRM-VEM and CCRM-VEM/VBL[30].[31] Structure **18b**, shown in **Figure 2.10** showed twofold lower activity against the mouse fibroblast cells compared to epothilone C.[32] Similarly, structure **19** shown in **Figure 2.10** showed lower activity against the human cervix cancer cells compared to epothilone D.[33]Although both structures **20** and **21** (**Figure 2.10**) contained an *(E)*-double bond at C-9-C-10 of epothilone ring reported significantly improved *in vivo* antitumor activity to their respective parent structures epothilone D and epothilone B in a mouse model of human breast cancer MX-1.

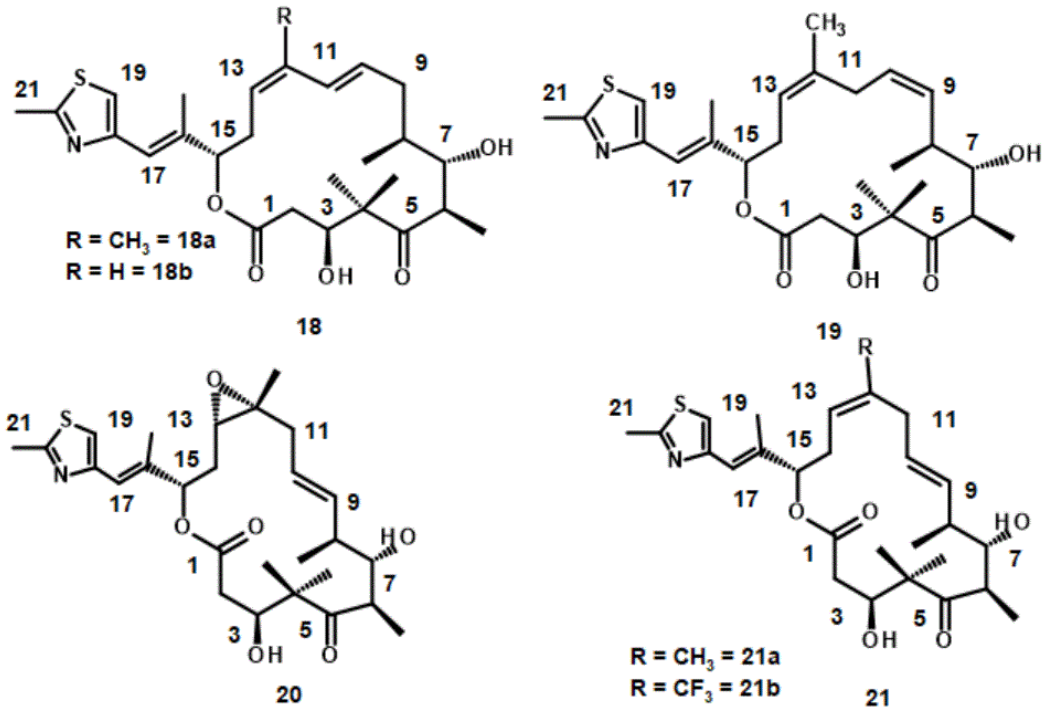


Figure 2.10: Modification of epothilone at C-12 and C-13

Interestingly, the structure **21a** also showed an enhancement of antiproliferative activity and improved plasma stability in mice model,[34],[35] but at the same time a clear enhancement in its toxic effect is observed.[35] Nevertheless, compounds shown in **Figure 2.10** were promoted to clinical development status. For structure **21b** of **Figure 2.10** similar anti-cancer cell tendencies were observed, but the compound was also associated with a significant increase in toxicity.[35]

Furthermore, another modification by incorporation of methyl at C-10 position of the macrolactone ring (*(S)*-10-methyl epothilone C proved to be detrimental for biological activity,[36],[37] whereas the furan at C-9-C-10 position proved to be tolerable for biological activity.[38]

Several laboratories have studied in detail the effects of modifications in the epoxide region of epothilone.[39] These modifications have led to the discovery of a number of potent epothilone analogues possessing whose biological activity more or less similar to that of epothilones A and B similar to that of epothilones A and B. Therefore, It has been shown that the epoxide moiety is not an absolute prerequisite for potent biological activity against cancer cells.

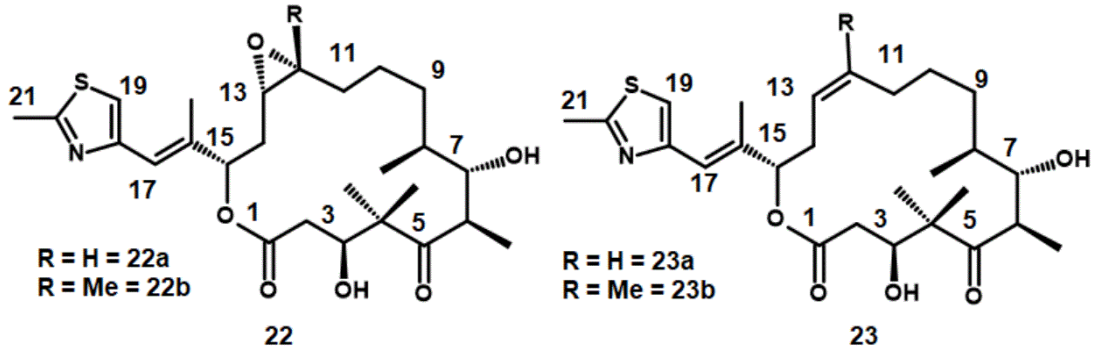


Figure 2.11: Structures of epothilone A-D

For example, epothilones C and D showed comparable biological activity compared to epothilone A and B, both epothilone C and D contain a *Z* double bond at C-12−C-13 position as shown in **Figure 2.11**, while they were slightly less active against human cancer cell growth compared to their parent epoxide molecules.[21],[14]

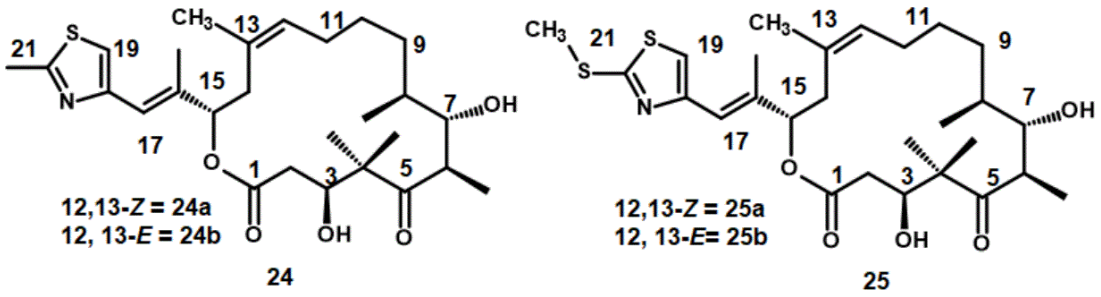


Figure 2.12: Modification of epothilone at C-13

In addition, a further modification was made in this region by placing the methyl group at C-13 position of the respective *Z* and *E* olefinic isomers. Structure **24a**, a *Z* configured olefinic isomer of **Figure 2.12**, showed 100-fold loss of activity compared to epothilone D. In contrast, *E* configured olefinic isomer **25b** showed the highest potency against these cancer cells.[40] Interestingly, Schering has reported *E* configured olefinic isomer with epoxide moiety in its patent, but did not publish any biological data. In further significant development, the C-13 olefinic bond was replaced by a tertiary amide. However, these structural modifications also resulted in a loss of activity against cancer cells compared to epothilone D. Similarly, the analogue haivng amides at C-13 position have also been reported as inactive compounds.[24]

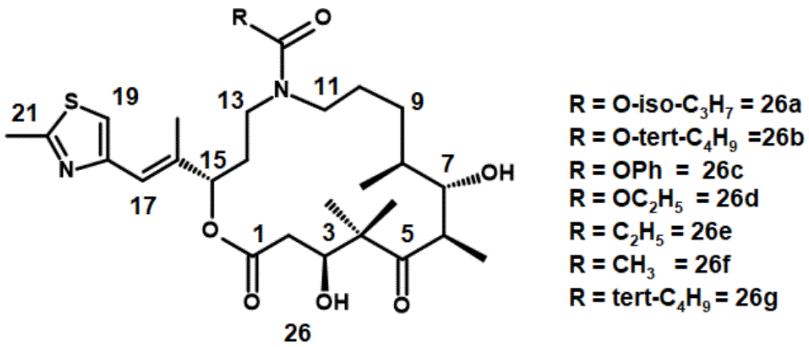


Figure 2.13: Carbamate modification of epothilone

Furthermore, Altmann and his coworkers prepared epothilone analogues by linking compounds such as carbamate and acyl with different alkyl chain, with nitrogen replacing the carbon in the C-12 position of epothilone ring (**Figure 2.13**). An interesting development was observed with compounds labeled azathilones (**26a-d**) which were reported to be less active cancer cell growth inhibitors compared to epothilone A or B,[41] but proved to be potent antiproliferative agents by exhibiting IC_{50} values between 30 nM and 300 nM against human cervix carcinoma cell line KB-31.[41]

Interestingly, however, some of these analogues were found to be significantly less active against the multidrug-resistant KB-8511 line compared to the drug-sensitive KB-31 parental line, leading to conclusion that analogues of compound **26** are generally better P-gp substrates than natural epothilones.

It is known that structural changes at the C-12-C-13 position are well tolerated as they have no negative effects on the biological activity of epothilones. Therefore, the new analogues were synthesized by the incorporation of compounds

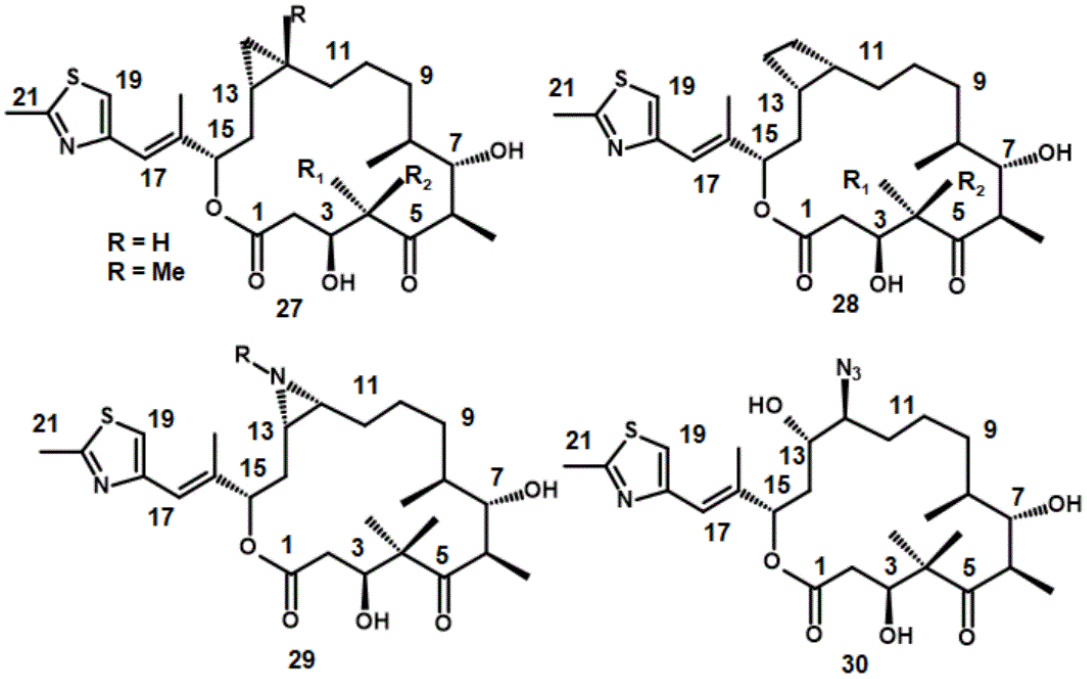


Figure 2.14: Modification at epoxy region of epothilone

such as cyclopropane, cyclobutane (**Figure 2.14**) and episulfide and aziridin analogues containing N-alkyl or N-acyl at the C-13 position and were found to be highly active. These modifications made it clear that the oxirane ring system in epothilones only serves to stabilize the correct bioactive conformation of the macrocyclic skeleton and does not act as a reactive electrophile or a hydrogen bond acceptor. The replacement of the oxirane ring in epothilones by a cyclopropane unit is therefore well tolerated and is not associated with a loss of tubulin-polymerizing or antiproliferative activity.[23]

Interestingly, it was found that azid-promoted epoxide ring cleavage with NaN_3 leads to azido alcohol as shown in **Figure 2.14** and that this analogue is significantly more potent against the human cervix cancer cell lines. In addition to the changes in the epoxide structure itself, modifications were made to the 26-methyl group in epothilones B or D by replacement of one of hydrogen atom of this methyl group with a substituent such as F, Cl, CH_3, or C_2H_5, RCH_2F,CH_2Cl, C_2H_5, n-C_3H_7 led to the development of the general rule that increasing the size of the substituent at the position of the 26-methyl group reduces biological activity, but some exceptions can also been found in literature.[23]

2.6 Modifications of Region D

The heterocyclic side chain of epothilones was frequent target for structural modifications. Although complete removal of heterocyclic ring resulted in a complete loss of activity.[42] However, Kingston and Horwitz proposed that the C-2 benzoyl residue of Taxol® and the thiazole side chain of epothilone occupy the same region of the protein.[43] By replacing of thiazole by pyridine, set a guiding principle that the position of nitrogen in the pyridine ring has to be exactly positioned in order to get it bonded with binding site of receptors by making it possible to serve as hydrogen bond acceptor.[44]

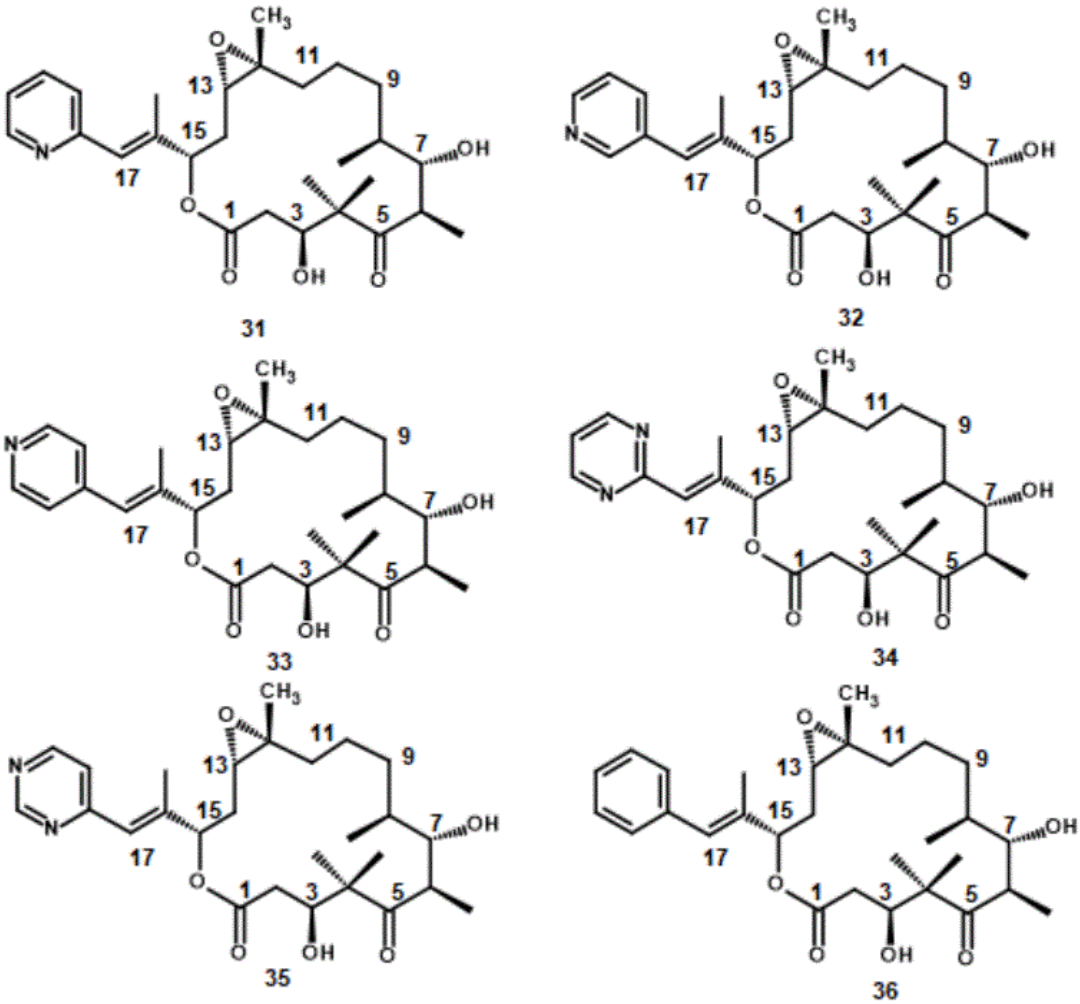


Figure 2.15: Modification at thiazole part of epothilone

The published data of structure **31** in **Figure 2.15** showed the highest activity

against cancer cells (85% tubulin polymerization and 0.30 by tubulin assay) because the nitrogen atome of pyridine ring is positioned ortho to the attachment point of the linker between the heterocycle and the macrocyclic skeleton,[24] whereas the transpositions of nitrogen atom of the pyridine, as shown by the structures **32** and **33** in the **Figure 2.15** led to loss of cellular activity compared to the structure **31**.[44]Even the incorporation of a second nitrogen atom, as shown in structure **34** (74% tubulin polymerization and 8.78 by tubulin assay) and structure **35** (47.3% tubulin polymerization and 14.9 by tubulin assay) also resulted in loss of activity, while the phenyl-derived analogue the structure **35** (28.0% tubulin polymerization and tubulin assay) were found to be even less potent.[44]

2.7 Total Synthesis of Epothilones

Epothilone A-F can be obtained through fermentation processes, but to perform the SAR studies, flexible and robust synthesis strategies had to be developed. Therefore, the first three syntheses (Danishefsky, Nicolaou, and Schinzer) were initially developed for epothilone A and epothilone B. These strategies are widely used for synthesis of new analogues. Therefore, these strategies are summarized in the following part of this chapter.

2.7.1 Danishefsky Syntheses [45-48]

Danishefsky *et al*. synthesized the side chain thiazole fragments **44** and **45** starting from thiazole ester **37**, which was reduced and oxidized to aldehyde **38**. Compound **39** was synthesized by a Wittig olefination of compound **38**. The resulting aldehyde (compound **39**) was enantioselectively converted to compound **41**.[45],[46],[47]

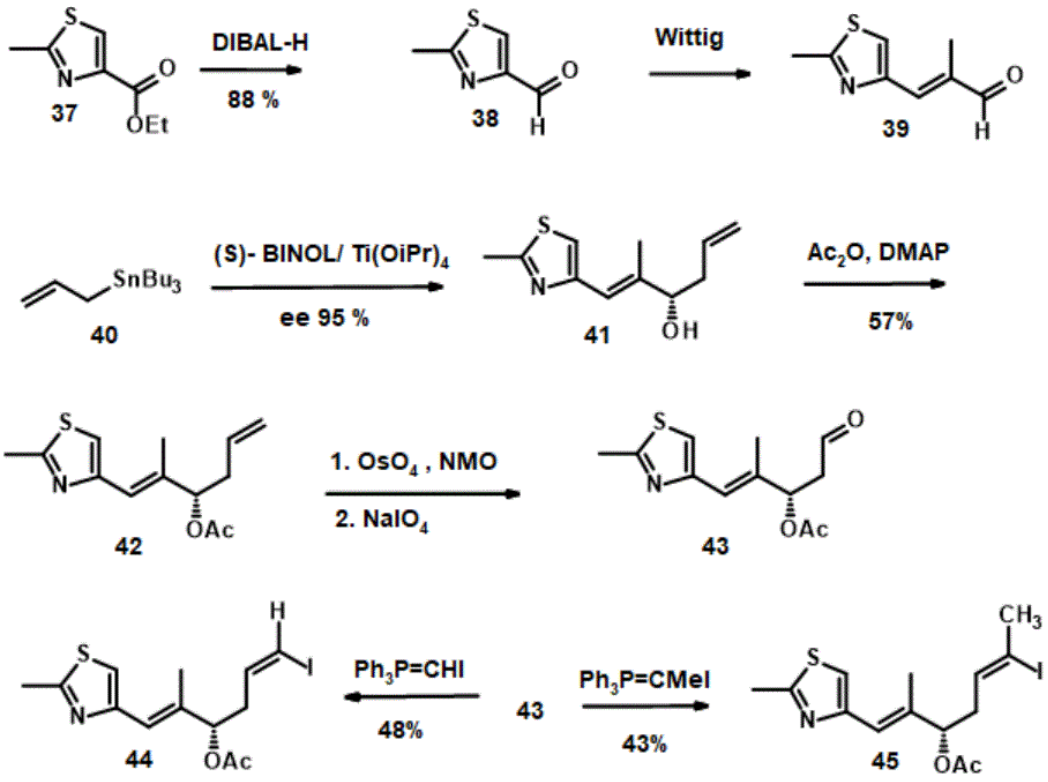


Figure 2.16: Danishefsky synthesis part 1

In the next steps, compound **41** was first protected (compound **42**) then oxidized (compound **43**) and olefinated to give compounds **44** and **45** (see scheme in **Figure 2.16**).

The C3-C11 segment (compound **53**) was prepared along a route in **Figure 2.17**. Thus, a Diels-Alder reaction between chiral aldehyde **46** and diene **47** was used to synthesize **48** with high Felkin-Anh model selectivity. The cyclopropanation was used to incorporate gem-dimethyl group at C4 in **49** and ring opening occured to iodide **50**. After deiodination, acyclic C3-C9 fragment **51** was obtained. Two successive extensions of one-carbon chain gave the C3-C11 fragment **53** via **52**. This compound **53** was converted to compound **54** and served as a precursor for Suzuki coupling.

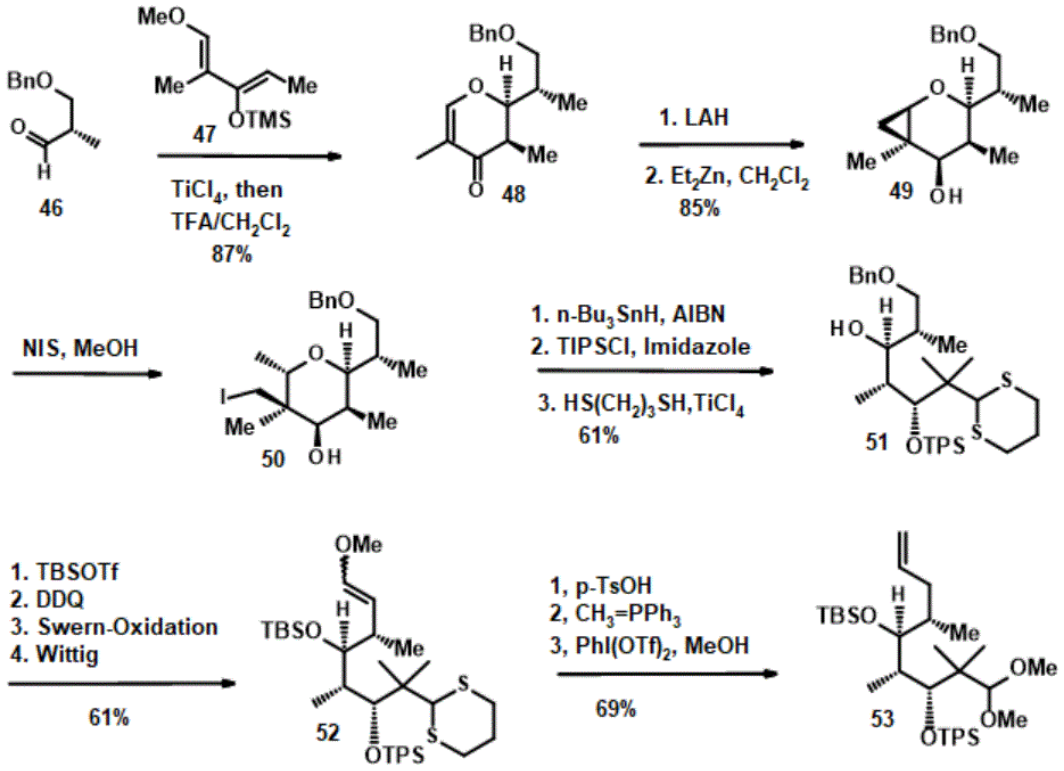


Figure 2.17: Danishefsky synthesis part 2

Thus, the Suzuki coupling between Compounds **54** and **44** led to the macroaldol precursor compound **55**. A simple aldol reaction then closed the ring to form compound **56** with a 6:1 diastereomeric ratio at C3. Then compound **56** was further deprotected, protected and oxidized at C5. The desilylation and stereoselective epoxidation led to form epothilone **22a** (see scheme in **Figure 2.18**).[48]

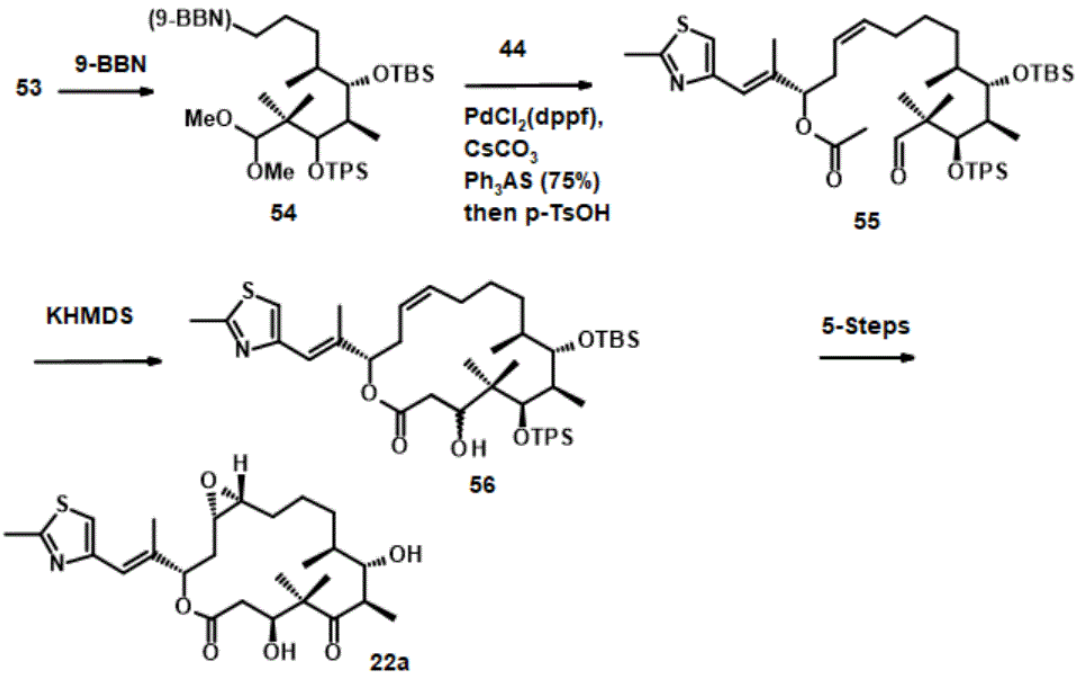


Figure 2.18: Danishefsky synthesis part 3

2.7.2 Nicolaou Syntheses [49-50]

The Nicolaou *et al*. synthesis strategy can be divided into two main classes: the ring-closing metathesis approach (first approach) and the ring closing macrolactonisation approach (second approach) for the epothilone A and C. In the ring closing metathesis approach, compound **58** was prepared by enantioselective allylation reaction.

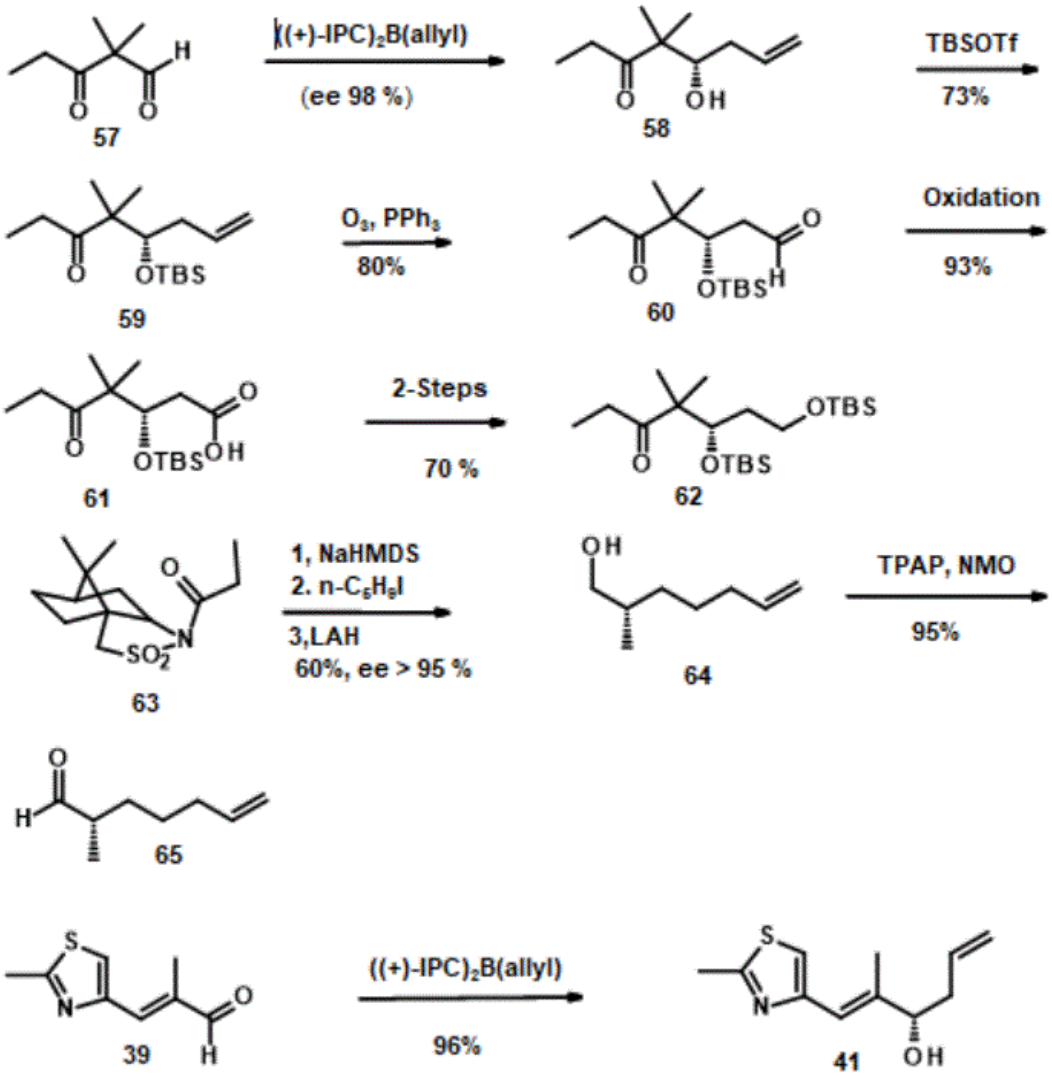


Figure 2.19: Nicolaou first approach for total synthesis of epothilone part 1

Subsequently, alcohol group of compound **58** was protected and the resulting compound **59** was oxidized by ozonolysis to produce the aldehyde, which was

further oxidized to compound **61** (carboxylic acid) as shown in **Figure 2.19**. Compound **61** was also reduced to a diol and protected to form compound **62**. Compound **62** is one of the key fragment to synthesize Epothilone B. While the stereocenter in side chain thiazole fragment **41** was prepared by the enantioselective allylation of compound **39** (see scheme in **Figure 2.19**).

As shown in **Figure 2.20** of the Nicolau synthesis, the compounds **66a** and **66b** were prepared by an aldol reaction between compounds **61** and **65**, both of which were esterified to compounds **67a**, **67b** and then diastereomer mixture was separated selectively in a ratio of 3:2. After the ring closing of compound **67a** by ring closing metathesis, it was deprotected and epoxidized to get epothilone A.[49]

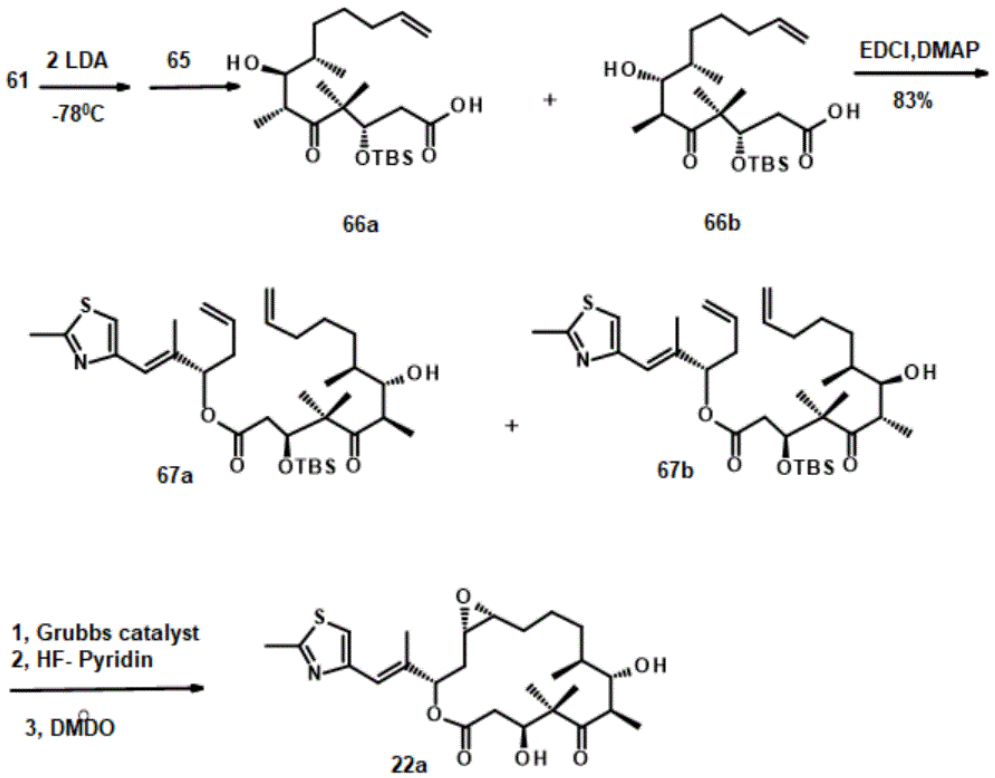


Figure 2.20: Nicolaou first approach for total synthesis of epothilone part 2

The second approach of Nicolaou is finalized by a macrolactonaization to cyclize epothilone molecule (see scheme in **Figure 2.22**). Compound **71** was synthesized by starting with enantioselective reduction of compound **39** and alcohol protection of compound **41**. The terminal double bond (compound **69**) was oxidized to the compound **70** as aldehyde. This aldehyde (compound **70**) is further converted to compound **71** as precursor for Wittig coupling (**Figure 2.21**).

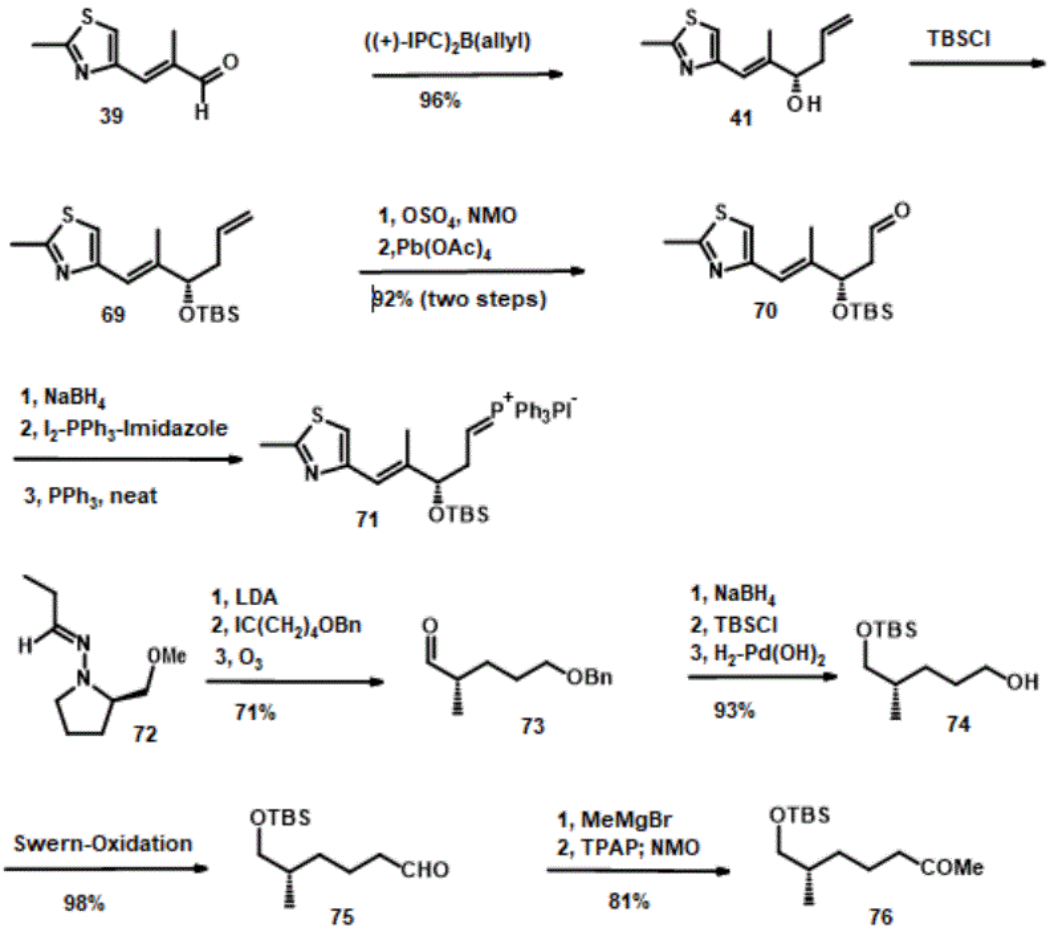


Figure 2.21: Nicolaou second approach for total synthesis of epothilone part 1

The required chiral aldehyde **75** as precursor for Wittig reaction was synthesized by converting compound **72** to compound **73**, which was reduced to alcohol then protected and subsequently selectively deprotected to yield compound **75** as shown by **Figure 2.21**. So the compound **71** via Wittig reaction was coupled with the aldehyde (compound **75**) resulting in the compound **77** containing double bond (**Figure 2.22**).

Deprotection of compound **77** and further oxidation to aldehyde (compound **78**) was accomplished by Swern oxidation. After aldol condensation between the compound **78** and compound **61**, resulting compound **79** was selectively deprotected at 15-OTBS and then esterified to give compound **80**. Then the protected secondary alcohols of compound **80** were deprotected with TFA, the double bond of the resulting compound was oxidized to form epothilone A (**22a**) as shown in **Figure 2.22**.[50]

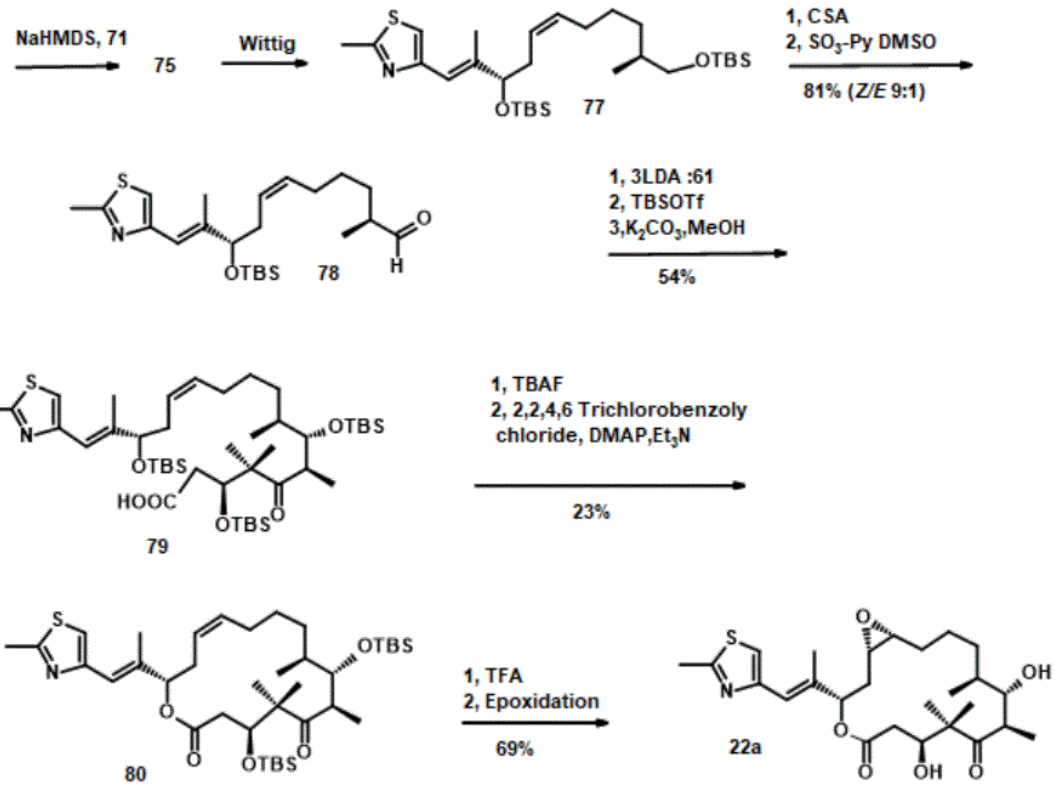


Figure 2.22: Nicolaou second approach for total synthesis of epothilone part 2

2.7.3 Schinzer Syntheses [51-52]

The required (*S*)- ethyl ketone **93** was synthesized by using the HYTRA-Route. (*S*)-2-Hydroxy-1,2,2-triphenylethylacetate [(*S*)-HYTRA)] **84** was synthesized by starting with mandelic acid esterification (**82**) followed by reduction with Grignard reagent to produce diol (**83**) and subsequent acylation yielded [(*S*)-HYTRA **84**)] as shown by scheme in **Figure 2.23**.

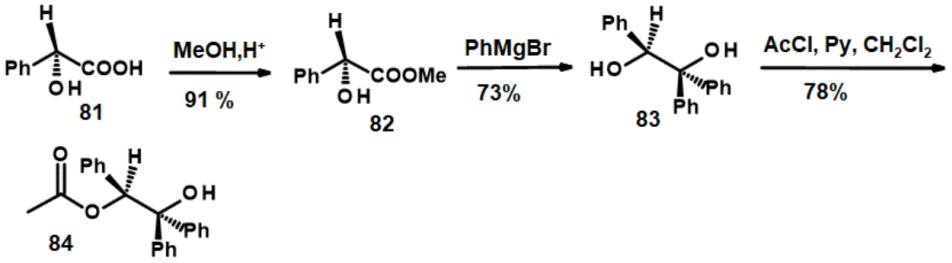


Figure 2.23: Schinzer synthesis part 1

The acetonide **93** was prepared by using a Reformatsky reaction as starting point for the preparation of ester (**86**) which was further dehydrated,reduced and oxidized to aldehyde (**89**) and then converted to compound **90** by using the Braun aldol addition.

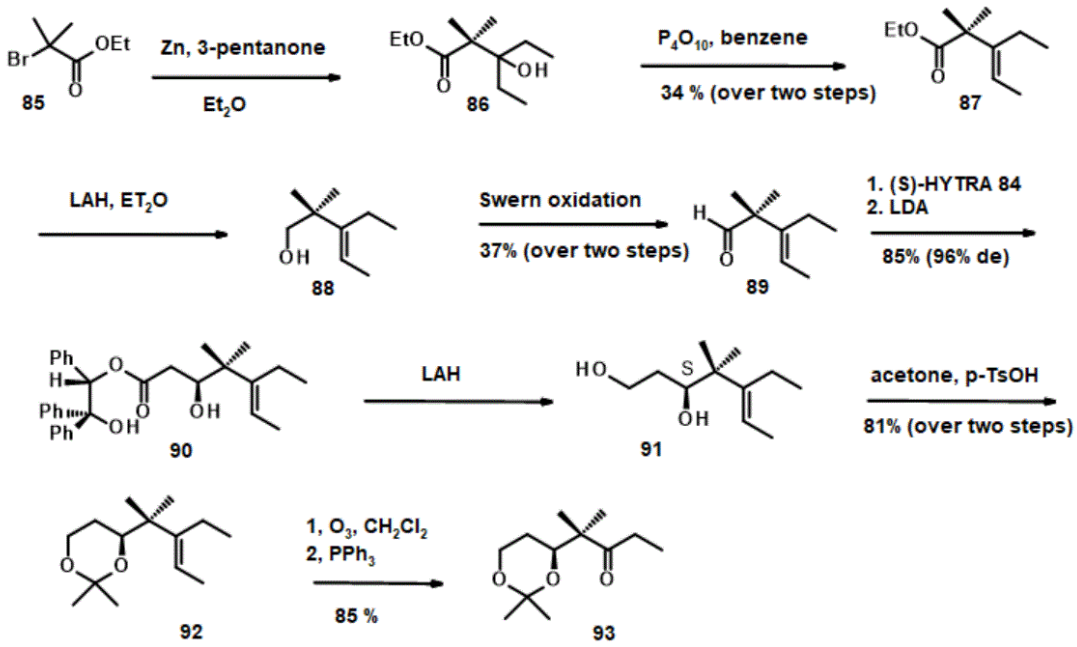


Figure 2.24: Schinzer synthesis part 2

This aldol product **90** was further reduced, protected and oxidized to get acetonide **93** as shown by **Figure 2.24**. For epothilone A, the key side chain thiazole fragment was synthesized starting by the reduction of compound **94** with Grignard reagent and followed with sharpless resolution. The resulting primary alcohol **96** was first protected, followed by oxidation of double bond to ketone (**98**) as shown in **Figure 2.25**. Compound **99** was produced via Wittig reaction and subsequently deprotected to yield compound **100**. Then compound **100** was further oxidized, olefinated and deprotected to produce thiazole fragment (**41**) of natural epothilone (see scheme in **Figure 2.25**).

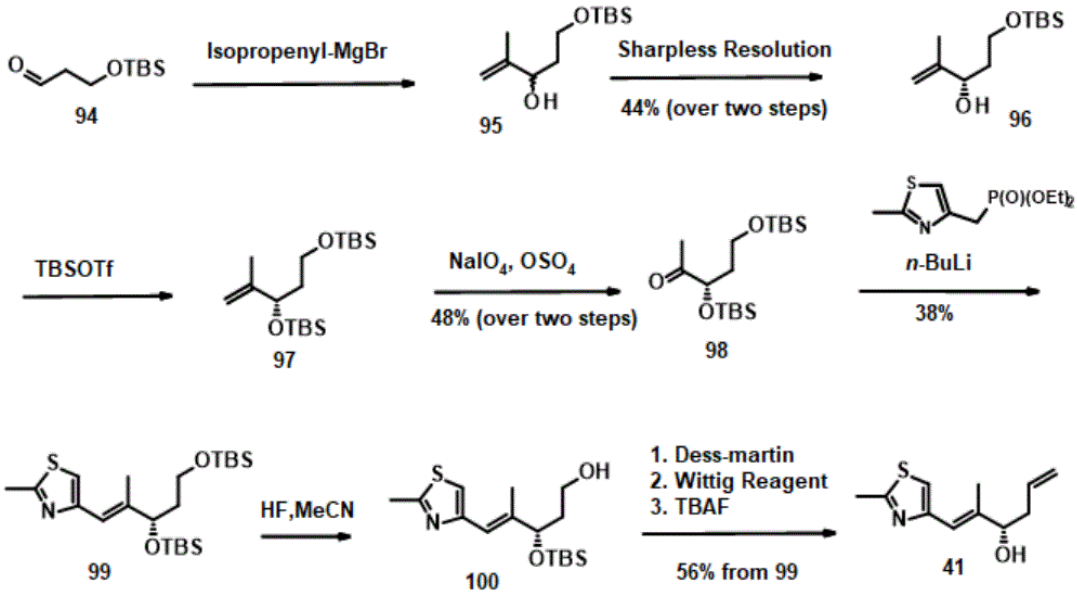


Figure 2.25: Schinzer synthesis part 3

Chiral aldehyde **65** was synthesized by following five steps starting with heptenoic acid **102** which was converted to acid chloride **103** and subsequent amidation yielded product **104**. The amide product was stereoselectively methylated to compound **105**, reduced and oxidized to furnish chiral aldehyde **65** (see scheme in **Figure 2.26**).

The acetonide **93** was then coupled with chiral aldehyde **65** via stereoselective aldol reaction to produce aldol product accompanied with higher stereoselectivity (see **Figure 2.26**). The aldol product **106a** was further converted into acid **110**. The alcohol **41** (thiazole side chain) and acid **110** were converted to di-olefin ester **111**. The ring closing metathesis was used for the cyclization of compound **111** and was finally deprotected to produce epothilone (see scheme in **Figure 2.26**).[51],[52]

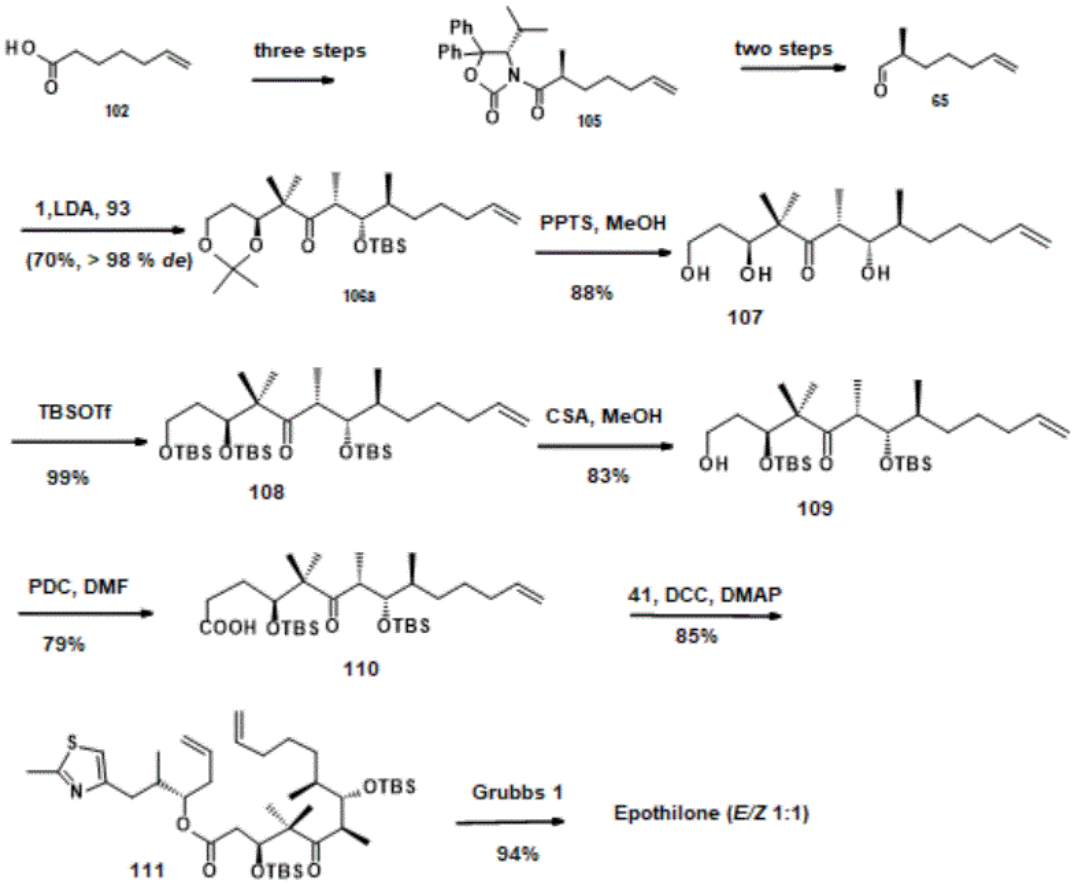


Figure 2.26: Schinzer synthesis part 4

Chapter 3

Aim of this work

The aim of this work is to develop the new analog of epothilone A by replacing thiazole fragment of the natural epothilone with an amino acid fused benzimidazole ring. The replacement of the thiazole fragment with the pyridine ring provided an interesting finding that the position of nitrogen atom in the pyridine ring is important for its biological activity.[44]

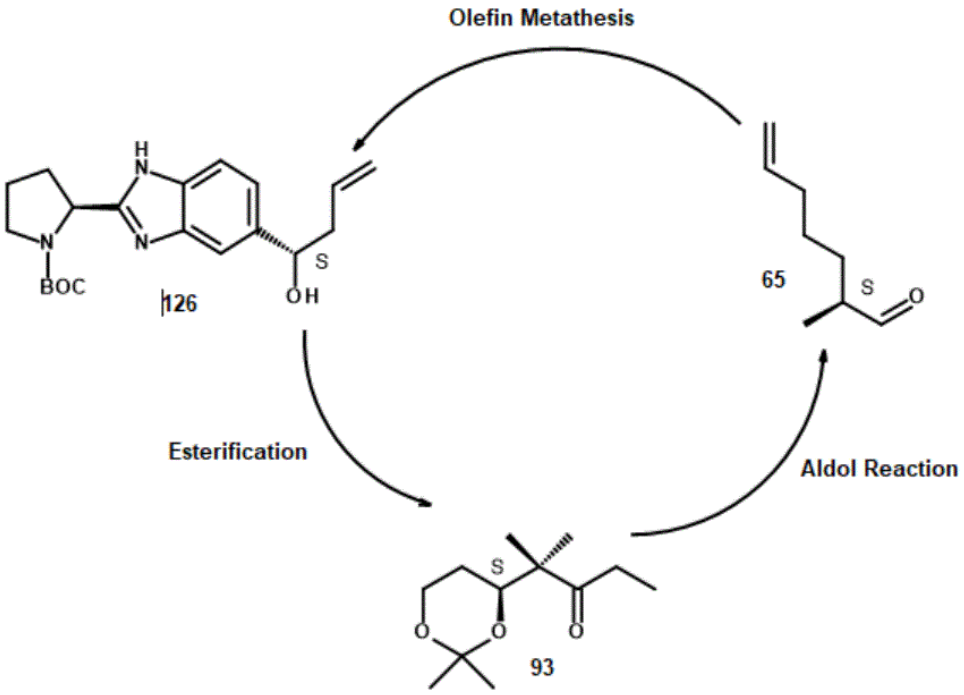


Figure 3.1 Synthesis strategy for the epothilone

Therefore based upon this fact, the thiazole fragment was replaced with the proline fused benzimidazole ring while the proline nitrogen protected with Boc group may enhance the activity of the epothilone. The whole synthesis strategy can be represented by the scheme in **Figure 3.1**: The Schinzer group has been working on total synthesis of epothilone since 1996 and his group had developed a very robust strategy that can be implemented for the synthesis of new epothilone analogues. The fragments **93**, **65** and **126** were synthesized and then coupled with each other by following the Schinzer strategy for the synthesis of new epothilone analogues.[44]

Chapter 4

Theoretical Section

4.1 Synthesis of (*S*)- Ethyl Ketone (93)

The chiral center of (*S*)- ethyl ketone **93** was synthesized by using a stereoselective aldol reaction between chiral ester enolate **84** [(*S*)-HYTRA)] and aldehyde **89**. The resulted β-hydroxyester **90** was reduced to diol **91** by using lithium aluminium hydride as reducing agent. Then diol **93** was first protected and subsequently ozonized to produce (*S*)-ethyl ketone **93** as shown in retrosynthetic scheme of **Figure 4.1**.

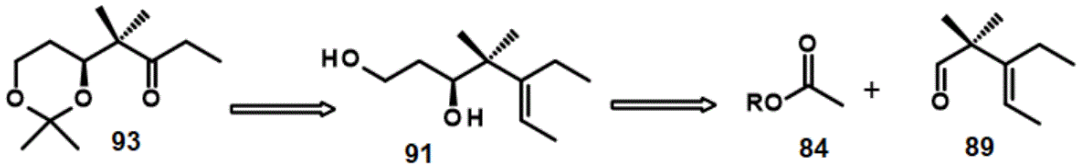


Figure 4.1: Retrosynthetic analysis of (*S*)- ethyl ketone **93**

4.1.1 Synthesis of (*S*)-HYTRA (84)

The chiral ester, also known as *(S)*-(-)-HYTRA **84** (1,1,2- triphenyl-1,2-ethanediol acetate) was synthesized in three steps via the synthetic route of Braun *et al*.[53] Braun's synthetic scheme shown in **Figure 4.2** started with *(S)*-(+)-mandelic acid **81** as a starting material. *(S)*-(+)-mandelic acid **81** was converted to ester **82** with a catalytic amount of sulfuric acid in the presence of methanol. Ester **83** was reduced to alcohol by addition of phenyl magnesium bromide to obtain diol **83** in 70% yield. The secondary alcohol of diol **84** was then acylated to

product **84** (71% yield) by using acetic anhydride in the presence of catalytic amounts of scandium (III) triflate (see the scheme in **Figure 4.2**),.[54] *(S)*-HYTRA was deprotonated and coupled by an aldol reaction with **84** and **89**.

The acylation step using scandium (III) triflate as a catalyst proved to be a more convenient and robust method compared to acylation step carried out by the Braun strategy.[54] In the modified acylation step, moisture free *(S)*-HYTRA **84** was easily obtained by using the direct filtration of the reaction mixture. In contrast to this newly developed strategy, in Braun's method to obtain *(S)* HYTRA **84** free from moisture was only achievable by heating under high vacuum for very long time (more than one week). The yield of product **84** by using scandium (III) triflate as a catalyst for the acylation of **83** was comparable to that of the Braun's methodology.

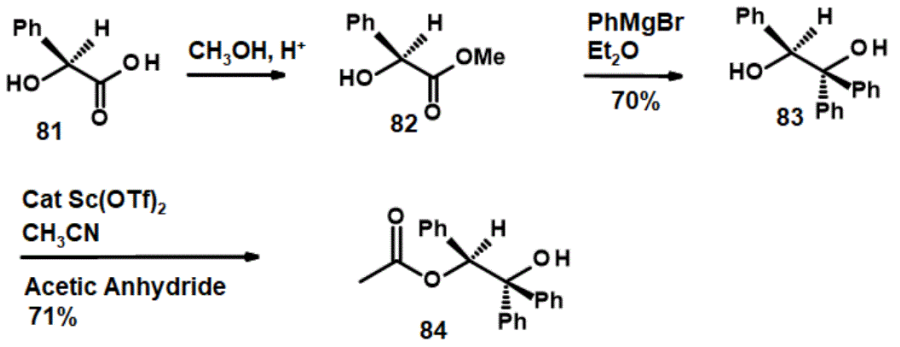


Figure 4.2: Scheme for chiral acetate **84** synthesis

4.1.2 Synthesis of Aldehyde (89)

The aldehyde **89** required for coupling with chiral ester **84** in an aldol reaction was prepared by using Reformatsky reaction with α-bromo ester **85** and 3-pentanone to give β-hydroxyester **86**. The Reformatsky reaction proceeded in 68 % yield. The dehydration of β-hydroxyester **86** was carried out using Sicapent (P_4O_{10}) in dry cyclohexane as solvent to give the olefinic ester **87** (82%, only the *(E)* isomer was obtained (detected by ^{1}H and ^{13}C NMR spectroscopy). This olefinic ester **87** was reduced with 3.5 mole equivalent of lithium aluminium hydride (LAH) as a reducing agent in dry Et_2O (diethylether) to alcohol **88** in 85 % yield. The alcohol **88** was then oxidized to aldehyde **89** in 72% yield using the Swern oxidation. The entire synthesis of the chiral aldehyde **89** is outlined in **Figure 4.3** .

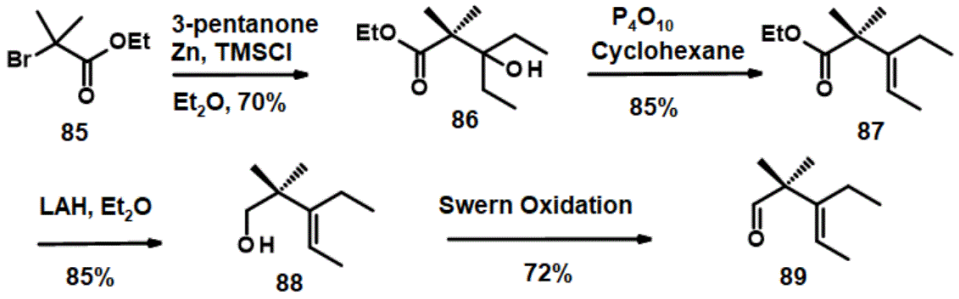


Figure 4.3: Scheme for aldehyde **89** synthesis

4.1.3 Aldol Coupling to Synthesize (*S*)- Ethyl Ketone (93)

The chiral acetate reagent since its first application has been widely used as a very useful tool in stereoselective aldol reactions for the syntheses of stereocenters in natural products and biologically active compounds. The structures of some of these compounds are shown in **Figure 4.4**.[55]Among these areγ-amino-β-hydroxybutanoic acid the enantiomeric naphthoquinones shikonin and alkannin, D- and L-digitoxose, desoxy and aminodesoxy furanosides, detoxinine, tetrahydrolipstatin, and related pancreatic lipase inhibitors, statin and statin analogues, compactin and mevinolin.[55]

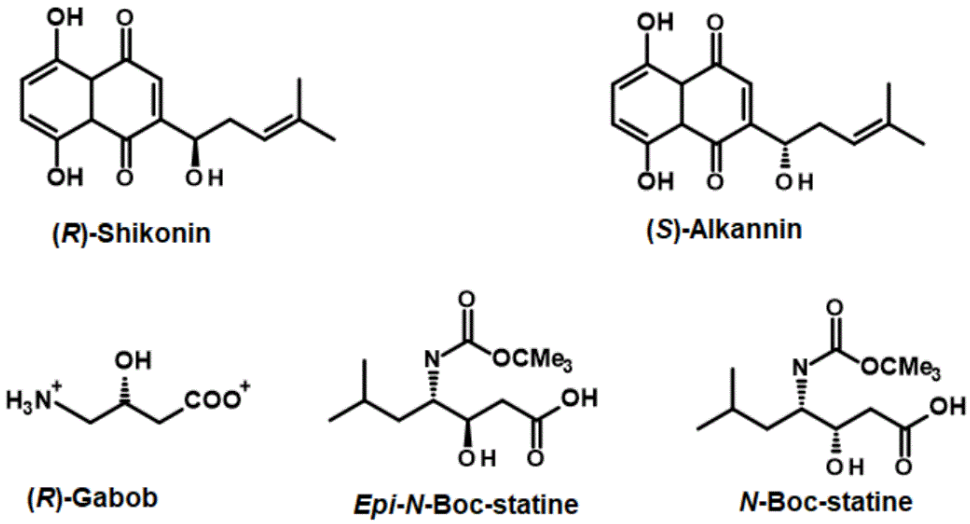


Figure 4.4: Use of (*S*)-HYTRA for the chiral center synthesis

It has been reported that the *(R)*-configured acetate always attacks the aldehyde predominantly from the *Re* side.

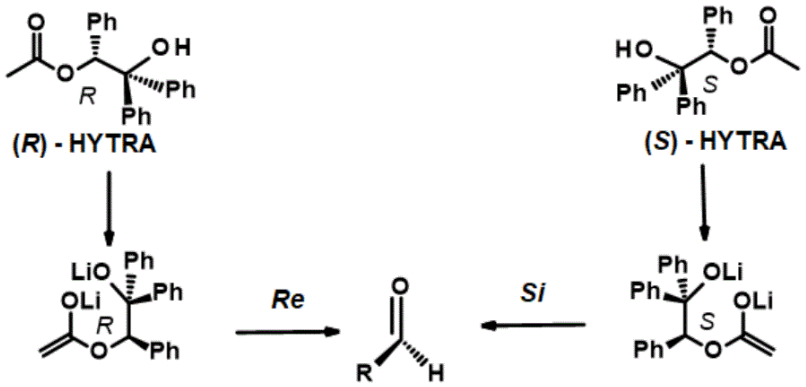


Figure 4.5: Stereochemical outcome of reaction between chiral ester **84** and aldehyde **89**

Whereas the *(S)*- configured acetate predominantly attacks from the *Si* side (see the mechanism in **Figure 4.5**). Thus, there is a predictable *lk* topicity in HYTRA aldol additions.[55]

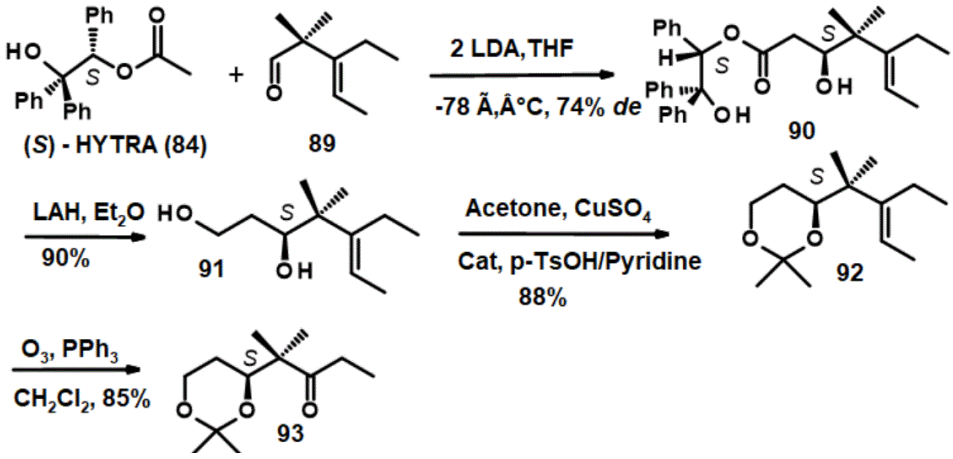


Figure 4.6: Synthesis of (*S*)- ethyl ketone **93**

In this regard, chiral acetate **84** reacts with aldehyde **89** to give the aldol product **90** with predominantly *(S)* configuration (74%, 94% *de*). The aldol product **90** was reduced with lithium aluminium hydride (LAH) to diol **91** in 90% yield and then the resulting diol **91** was protected with dry acetone and anhydrous $CuSO_4$

in presence of *p*-TsOH and pyridine as catalyst yielding the acetonide **92** in 88% yield. Finally, ozonolysis gave the desired (*S*)- ethyl ketone **93** in 85% yield. This entire strategy can be represented by the scheme shown in **Figure 4.6**. It is noteworthy to say that this strategy for the preparation of the chiral center at C-3 position of epothilone ring proved to be a very robust and reproducible methodology.

4.2 Synthesis of Chiral Aldehyde (65)

The synthesis of *(S)*-methylhept-6-enal **65** was carried out according to the Evans methodology to establish stereocenter at the α position of aldehyde **65** in five steps by induction of methyl group.[56],[57] The retrosynthetic analysis for the chiral aldehyde synthesis is shown in synthesis scheme of **Figure 4.7**.

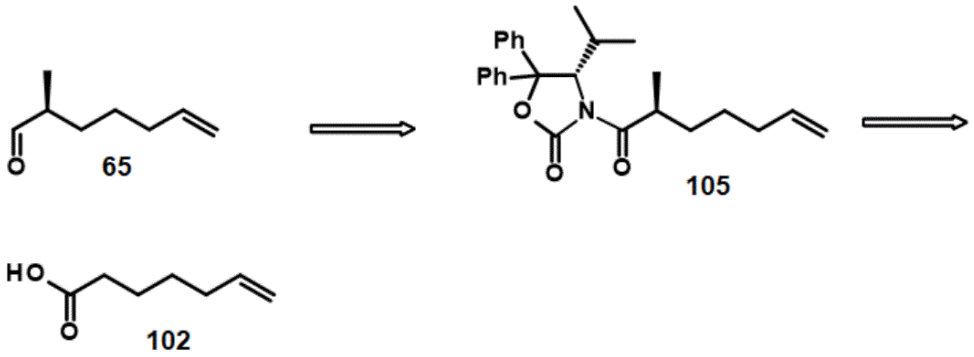


Figure 4.7: Retrosynthetic analysis of chiral aldehyde **65**

For the synthesis of chiral aldehyde **65**, it was necessary to first synthesize the Evans auxiliary [(*S*)-4-Isopropyl-5,5-diphenyl-oxazolidin-2-on **101**]. The Evans auxiliary **101** was synthesized by using the protocols mentioned in experimental section.

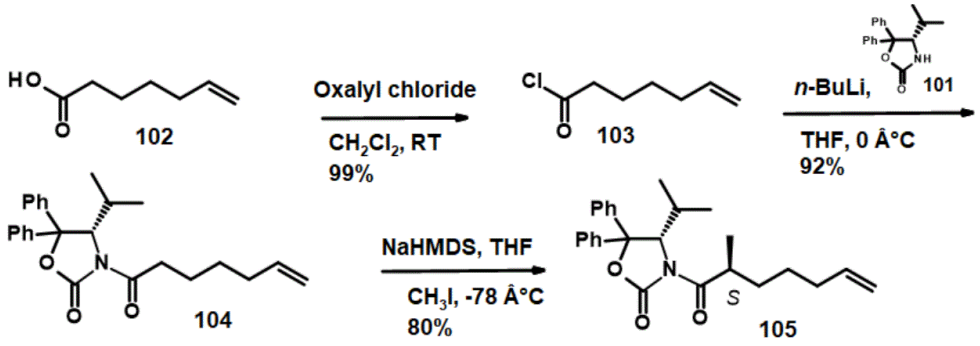


Figure 4.8: Synthesis of chiral aldehyde **65** part 1

The Evans auxiliary was first coupled with 6-heptenoic **102** acid via its acid chloride. The acid chloride **103** was synthesized in a very good quantitative yield using oxalyl chloride in dry dichloromethane. As shown in synthesis scheme in **Figure 4.8** acid chloride **103** was then coupled with Evans auxiliary **101** (oxazolidinone) using *n*-BuLi as base, resulting in amide product **104**. The amide product **105** was deprotonated at α position using NaHMDS as base in dry THF at -78 °C by chelating the Na^+ cation with the counter oxygen anion as shown in **Figure 4.9**.

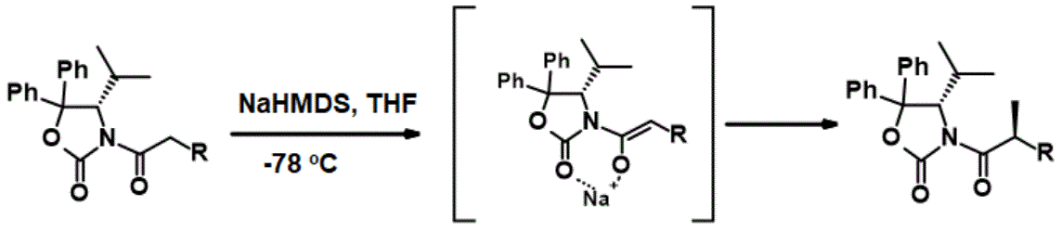


Figure 4.9: Chelation mechanism of Evans auxiliary

This intermediate was selectively methylated at α position by using MeI as a methylating agent (see **Figure 4.10**). In short, the overall synthetic strategy for the preparation of aldehyde **65** proved to be very robust and selective for successful synthesis of the (*S*)-configured aldehyde.

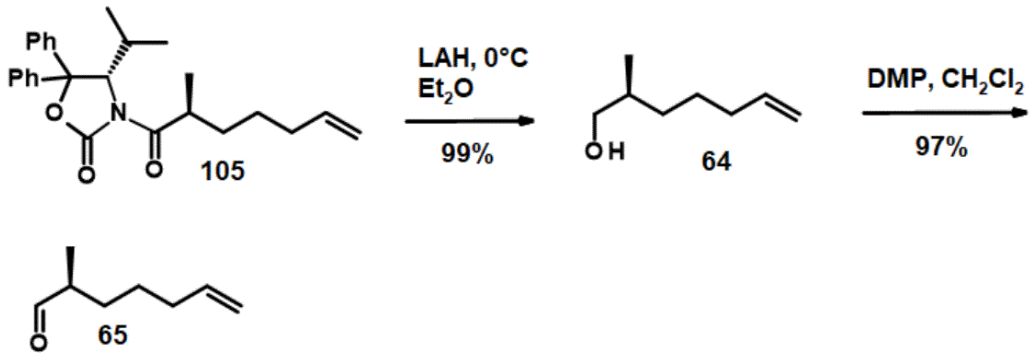


Figure 4.10: Synthesis of chiral aldehyde **65** part 2

4.3 Aldol Coupling between (*S*)- Ethyl Ketone (93) and Aldehyde (65)

Enolate formation in the aldol reaction is simply an acid base reaction and is an important factor in determining whether the reaction is kinetically or thermodynamically controlled. The equilibrium position is generally controlled by several factors such as solvent chosen, the choice of a stronger or weaker base, the cation chosen, and the temperature at which the reaction takes place.[55] The factors that control the kinetic outcome of the reaction are an aprotic solvent such as Et_2O, THF, a strong base such as LDA which produces a weak conjugate acid, an oxophilic cation such as Li^+, and a temperature of -78 °C.[55]

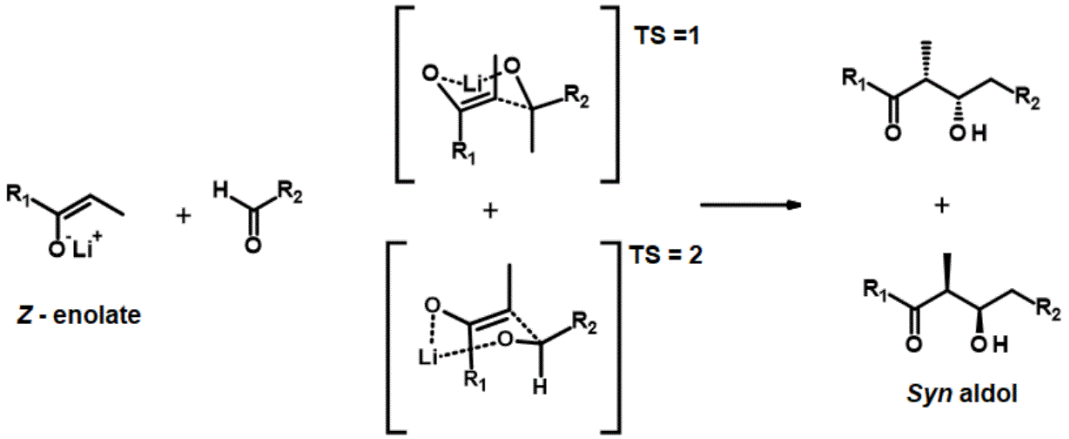


Figure 4.11: Reaction mechanism of (*S*)- ethyl ketone **93** with aldehyde **65**

The stereoselective reaction between (*S*)- ethyl ketone **93** and the chiral aldehyde **65** which proceeds under kinetic conditions and its outcome can be rationalized by Zimmerman-Traxler transition state hypothesis model shown in **Figure 4.11**.[58] The model states *Z*-enolate primarily generates *syn*-aldol, whereas *E*-enolate leads to the formation of *anti*-aldol products. The chair like conformation of transition states shown in **Figure 4.11** occurs when *Z*-enolate of lithium coordinates with oxygen atoms of both aldehyde and enolate. In both type of transition states, the substituent of aldehyde preferentially occupies the equatorial position.[55]

In this methodology, a *Z*-enolate was selectively generated by deprotonation of (*S*)- ethyl ketone with LDA at -78 °C in THF, to which chiral aldehyde was added at -78 °C. In this reaction, the bulky substituent of (*S*)- ethyl ketone and the steric hindrance of chiral aldehyde produced a C7-C8 *anti*-product (*anti*-Cram product) corresponding to the natural epothilone ring configuration and C7-C8 *syn*-product (Cram product) in a ratio of 10:1(see **Figure 4.12**).

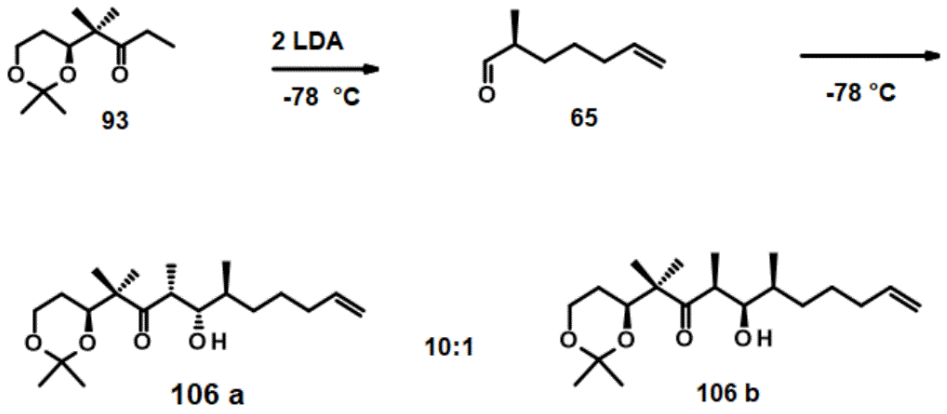


Figure 4.12: Aldol reaction between chiral ketone **93** and chiral aldehyde **65**

4.4 Synthesis of Acid of Macroaldol Product (110)

The aldol product **106a** was converted to **110** according to the known protocols of Schinzer *et. al* but with some necessary modifications to improve the cumulative yield of the synthetic strategy (see reactions scheme in **Figure 4.13**).[51]

In the first step, the acetonide cleavage of the aldol product **106a** was carried out using cerium chloride as a cleaving agent. This cleavage reaction proceeded very cleanly and gave the triol **107** with 84% yield without any by-product.

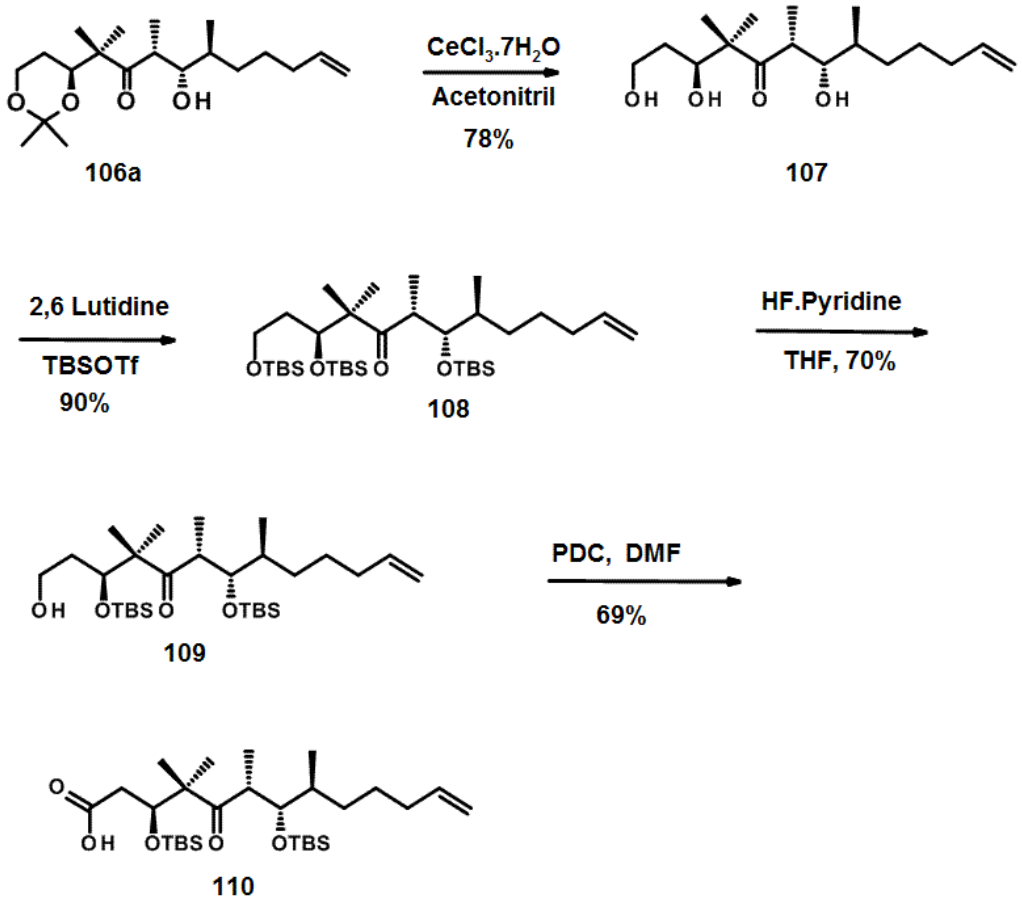


Figure 4.13: Synthesis strategy of macroaldol product **110** of epothilone

The alcohol groups of this triol aldol product **108** were protected with TBSOTf and 2,6 Lutidine as base. The protection of the alcohols groups of **107** gave the product **108** in 90% yield. In the next step, the primary TBS-ether of the

aldol product **108** was selectively deprotected using pyridinium-HF as cleavage reagent. This reaction deprotected the primary TBS-ether and gave **109** in 88% yield. In the last step, compound **109** was oxidized with PDC in DMF as reagent to obtain the acid **110**. This step led to the acid aldol product **110** in 78 % yield.

4.5 Synthesis of Benzimidazole Fragment (126)

Due to their importance for the pharmaceutical industry, the synthesis of benzimidazole rings has been published in various publications.[60],[61],[62],[63] The retrosynthetic analysis of the enantioselective benzimidazole ring **126**, the fragment required for coupling with aldol product **110** of epothilone ring, is shown in **Figure 4.14**.

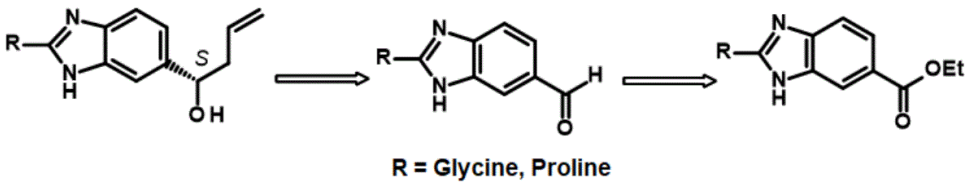


Figure 4.14: Retrosynthetic analysis of enatioselective benzimidazole fragments

The synthesis began with the synthesis of amino acid derived benzimidazole ester rings **121** and **123**, which were subsequently converted to benzimidazole aldehydes **125**, **128** by reduction and oxidation. These aldehydes were enantioselectively reduced by Brown allylation to generate the required alcohols **126** and **129**, which were intended to replace the thiazole ring **41** of the natural epothilone.

4.5.1 Synthesis of Benzimidazole Ester (121) and (123)

To achieve this goal, various oxidative or reductive cyclization strategies are available with which benzimidazole rings fused to amino acids can be synthesized. However, the classical method to construct a benzimidazole ring is the dehydration of the amide under harsh conditions, e.g. using a strong acid and carrying out the reaction at elevated temperature. These possible synthesis strategies are shown in **Figure 4.15**.

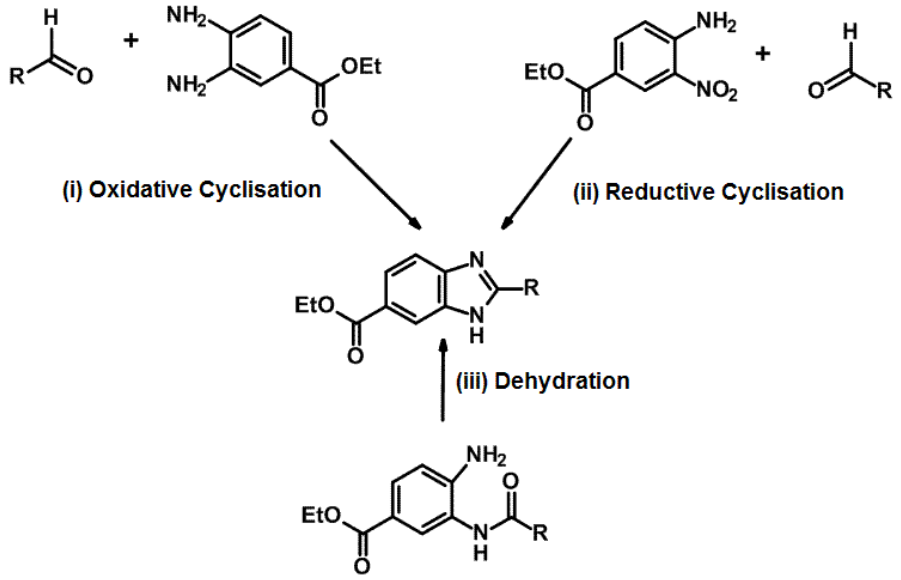


Figure 4.15: Synthesis strategies of benzimidazole rings **121** and **123**

The synthesis of aldehyde derived from respective amino acid is shown in reaction scheme in **Figure 4.16**. The Boc-L proline **112** and Boc glycine **115** were converted to corresponding Weinreb amide **113** and **116** using EDCI, DMAP as coupling agents, giving 85% and 80% yields respectively. The Weinreb amide products **113** and **116** were reduced with LAH in dry diethyl ether as solvent at 0 °C to afford aldehydes **114** and **117**. The reactions gave 75% and 80% yields respectively.[64] The resulting aldehydes **114** and **117** were condensed with ethyl 3,4-diaminobenzoate **118** under the oxidative conditions to generate benzimidazoles **121** and **123** (see scheme in **Figure 4.17)**[60]

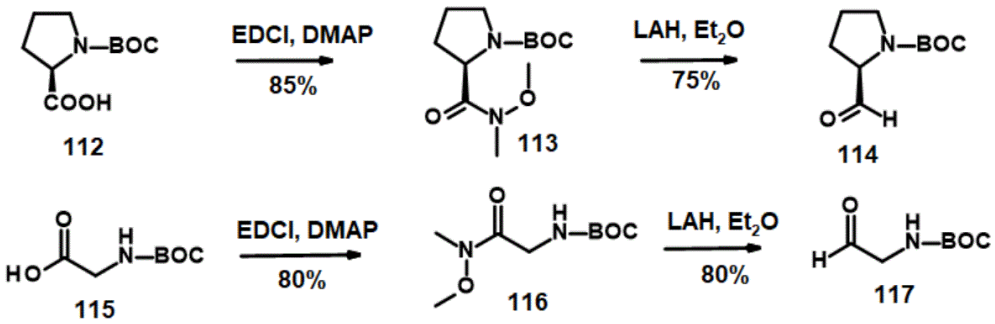


Figure 4.16: Synthesis strategy of Boc-L-prolinal **114** and Boc-glycinal **117**

Similarly **114** and **117** were also coupled with ethyl 4-amino-3-nitro-benzoate

119 under the reductive condition to generate benzimidazole esters **121** and **123**.[61] These reactions produced benzimidazoles esters in very low yields.

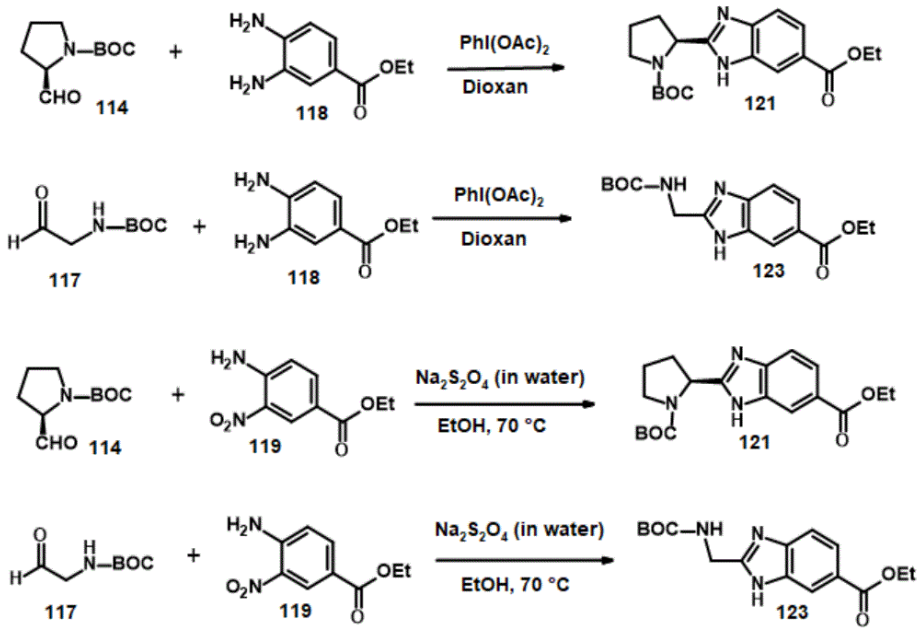


Figure 4.17: Synthesis of benzimidazole-ester **121** and **123** from aldehydes

For the dehydration of the amides (**120** and **122**), a new synthetic strategy was used in which the reaction is carried out under milder rather than harsher conditions to synthesize the benzimidazole ester rings **121** and **123** (see **Figures 4.18 and 4.19)**.

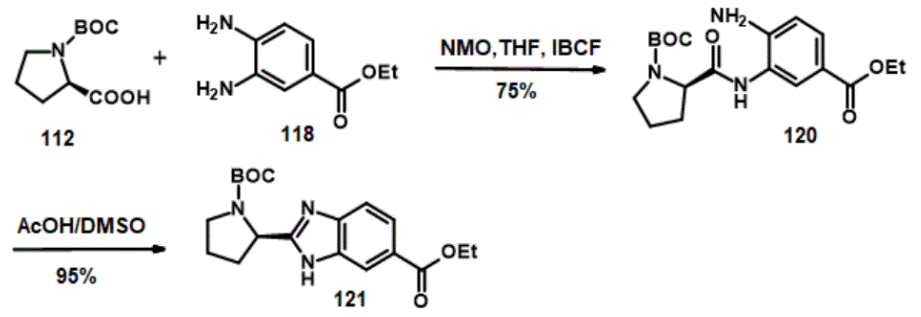


Figure 4.18: Synthesis scheme of benzimidazole-ester **121** from amide **120**

In this strategy, the amide **120** was synthesized in 75% yield using mixed anhydride reaction conditions between the reactants Boc-L-proline **112** and

ethyl 3,4-diaminobenzoate **118**. Similarly, the amides **122** was also synthesized in 85% yield using mixed anhydride reaction conditions to couple Boc-glycine **115** and ethyl 3,4-diaminobenzoate **118**.[64]The resulting amides **120** and **122** were dehydrated with acetic acid and dimethyl sulfoxide (DMSO) in a 3:1 ratio and the reaction mixture was heated at 65 °C for 12 hours.[62] Dehydration of amides **120** and **122** gave the benzimidazole esters **121** and **123** in excellent yields.

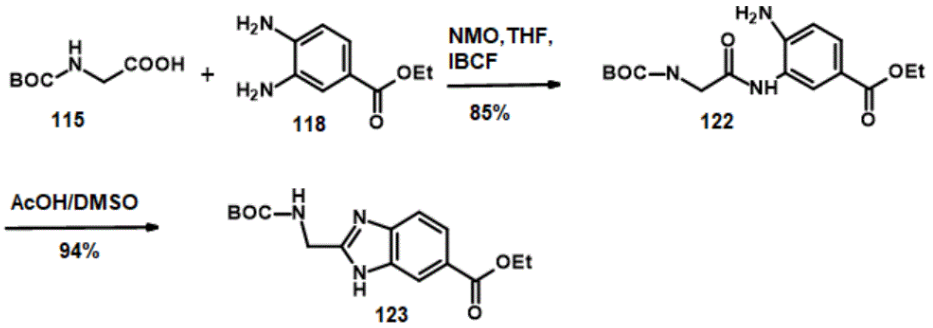


Figure 4.19: Synthesis scheme of benzimidazole-ester **123** from amide **122**

Table 4.1 below summarizes the overall results of the cyclization study. The data to obtain the desired benzimidazole ester result showed that the amide dehydration method proved to be the best in terms of yield.

Reactant 1	Reactant 2	Cyclisation condition	Time	Yields
Ethyl 3,4-diaminobenzoate **118**	Aldehyde **114**	Oxidative	12 hr	8%
Ethyl 4-amino-3-nitro-benzoate **119**	Aldehyde **117**	Reductive	12 hr	4%
Ethyl 3,4-diaminobenzoate **118**	Aldehyde **114**	Oxidative	12 hr	7%
Ethyl 4-amino-3-nitro-benzoate **119**	Aldehyde **117**	Reductive	12 hr	10%
Amide **120**	AcOH/DMSO	Dehydration	12 hr	94%
Amide **122**	AcOH/DMSO	Dehydration	12 hr	95%

Table 4.1: The synthesis of benzimidazole **121** and **123** a comparison of yields

4.5.2 Synthesis of Benzimidazole Allyl Alcohols (126) and (129)

The benzimidazole ester **121** and **123** had to be converted to the (*S*)-configured benzimidazole allyic alcohol fragments **126** and **129** which were required to couple with the acid **110** to form an ester **130**. The enantioselective synthesis of **126** and **129** was carried out using Brown allylation method. Brown allylation proved to be very robust method for producing the desired allylic alcohols with an enantiomeric excess (e.e) of 9:1 (see **Figure 4.20**).

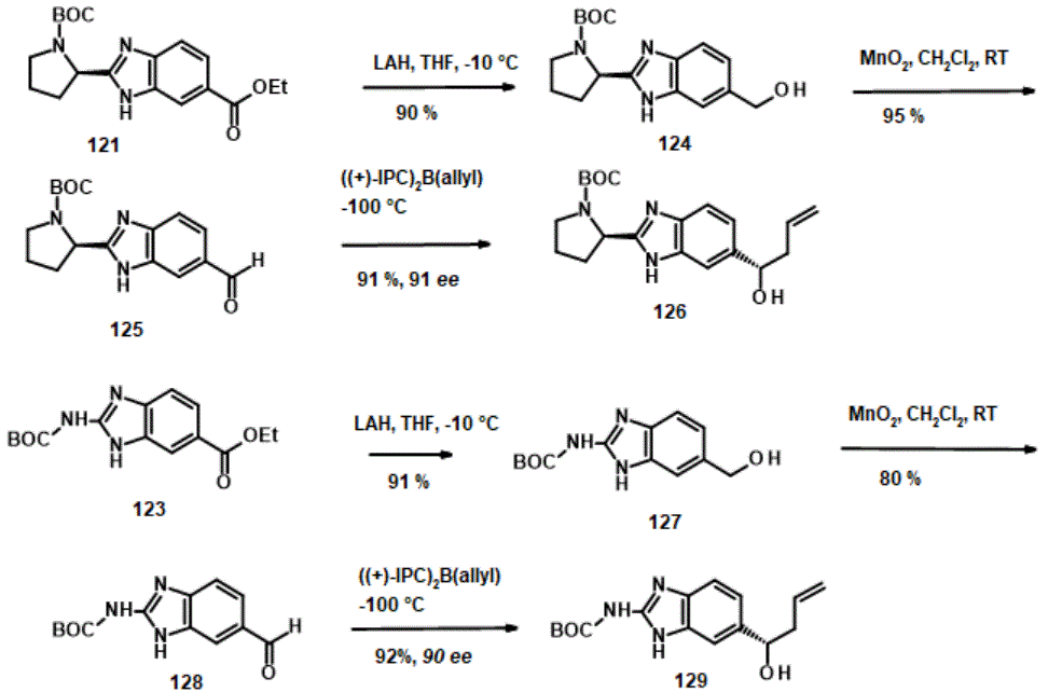


Figure 4.20: Synthesis scheme of benzimidazole allyl alcohols **126** and **129**

The reactions started with the reduction of esters, therefore, the benzimidazole esters **121** and **123** were reduced in dry diethylether with LAH as reducing agent in 70% yield to benzimidazole alcohols **124** and **127**. The benzimidazole alcohols **124** and **127** were oxdised in 74% yield in dry dichloromethane to give the aldehydes **125** and **128**. The benzimidazole aldehyde was then reduced enantioselectively to *S* configured benzimidazole allyl alcohols (**126** and **128**) by using Brown allylation methodology. The Brown allylation led to products **126** and **129** in 91% and 92% yield respectively.[65] This overall synthetic strategy is shown by the scheme in **Figure 4.20**.

4.6 Synthesis of Metathesis-substrate (130)

The benzimidazole allylic alcohol fragment **126** was coupled with acid **110** using EDCI/DMAP reagents for the esterification. This reaction gave the metathesis-substrate **130** in 80% yield (see **Figure 4.21**).

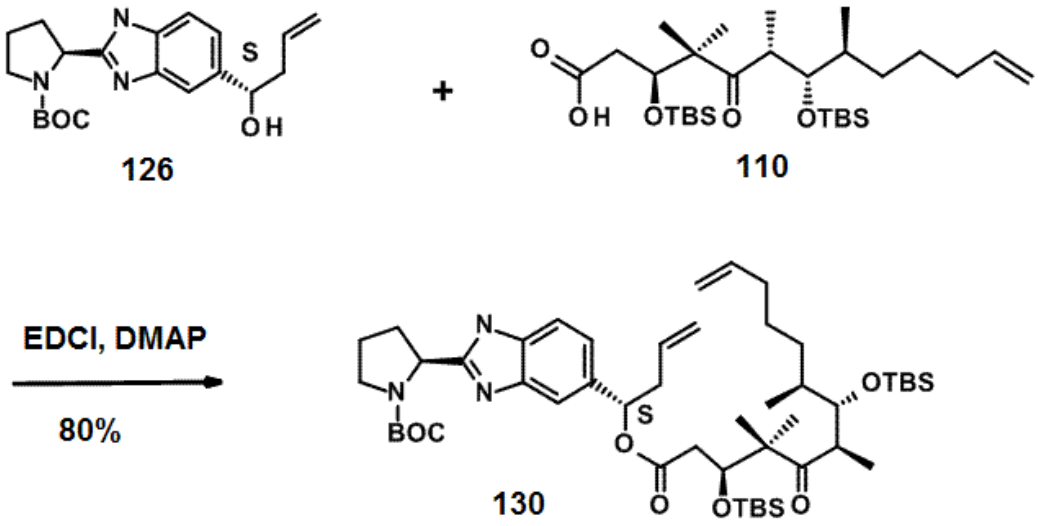


Figure 4.21: Synthesis of epothilone metathesis-substrate

4.7 Ring closing of Metathesis-substrate (136)

A major problem in the total synthesis is that the enormous number of linear reactions required result in a dramatic decrease in overall yield. The ability of metathesis to selectively lead to a desired biologically active stereoisomer or enantiomer makes it an efficient approach that can considerably improve the outcome of otherwise difficult natural product syntheses.[66] The metathesis reaction can be divided into three main groups:

- ring-closing metathesis
- cross-metathesis
- ring-opening metathesis

4.7.1 Ring-closing Metathesis

Alkene ring-closing metathesis has developed into one of the most powerful and reliable methods for ring formation in the total synthesis of the natural products. The Danishefsky, Schinzer and Nicolaou groups used ring closing metathesis for the synthesis of the epothilones. Danishefsky used Schrock catalyst for ring closing metathesis, whereas Nicolaou and Schinzer used Grubbs 1 catalyst for ring closing metathesis.

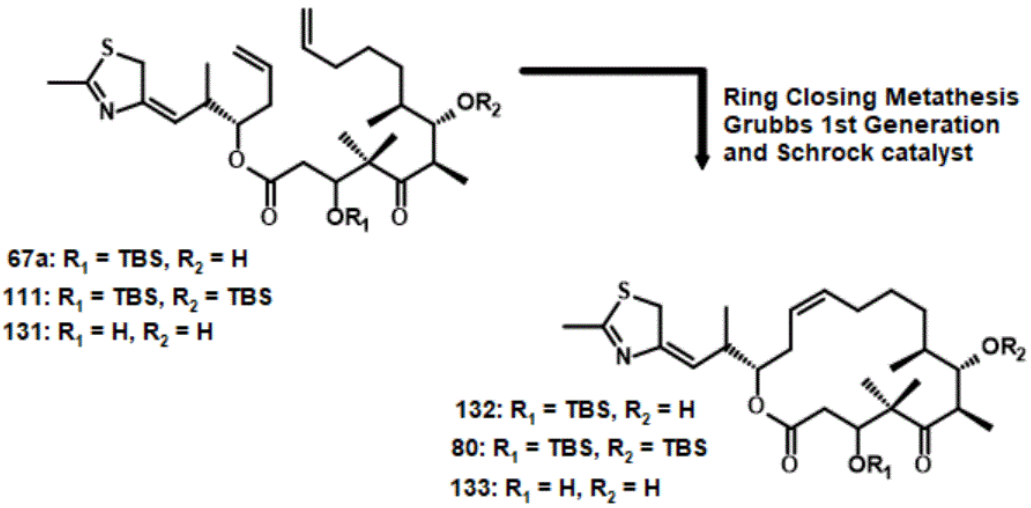


Figure 4.22: Ring closing metathesis

At that time not only functional compatibility of the thiazole fragment but also unprotected alcohol groups were questionable about the stereochemical outcomes of the metathesis. The ring-closing metathesis strategy used by Danishefsky, Nicolaou and Schinzer for various substrates with different functionalities and the corresponding reaction conditions are summarized in **Table** 4.2.[67]

Nicolaou *et. al*	Danishefsky *et. al*	Schinzer *et. al*	Danishefsky *et. al*
(67a-132)	(111-80)	(111-80)	(131-133)
(10 mol %)	(50 mol %)	(6 mol %)	(50 mol %)
CH_2Cl_2, 25 °C	Benzene, 55 °C	CH_2Cl_2, 25 °C	Benzene, 55 °C
(85% *E/Z* 1:1.2)	(86% *E/Z* 3:5)	(94% *E/Z* 3:5)	(65% *E/Z* 3:5)

Table 4.2: Overview of RCM strategy for epothilone synthesis

Schinzer and coworkers used and optimized ring closing metathesis as a very useful strategy for the synthesis of the C12-C13 double bond of the epothilone

ring. Since ring closing metathesis has become a very powerful tool for alkene synthesis in organic synthesis, the Grubbs catalyst was modified to improve its tolerance and efficiency. The second-generation Grubbs catalyst is an improved version of the first-generation catalyst that is tolerant to the presence of moisture in the reaction and can also be used with the sterically hindered terminal alkene bonds. Therefore, the second generation Grubbs catalyst was used for ring closing of the metathesis substrate **130**. The reaction mixture in toluene was refluxed for two hours resulting in a mixture of *Z* and *E* isomers in 1:1 ratio.

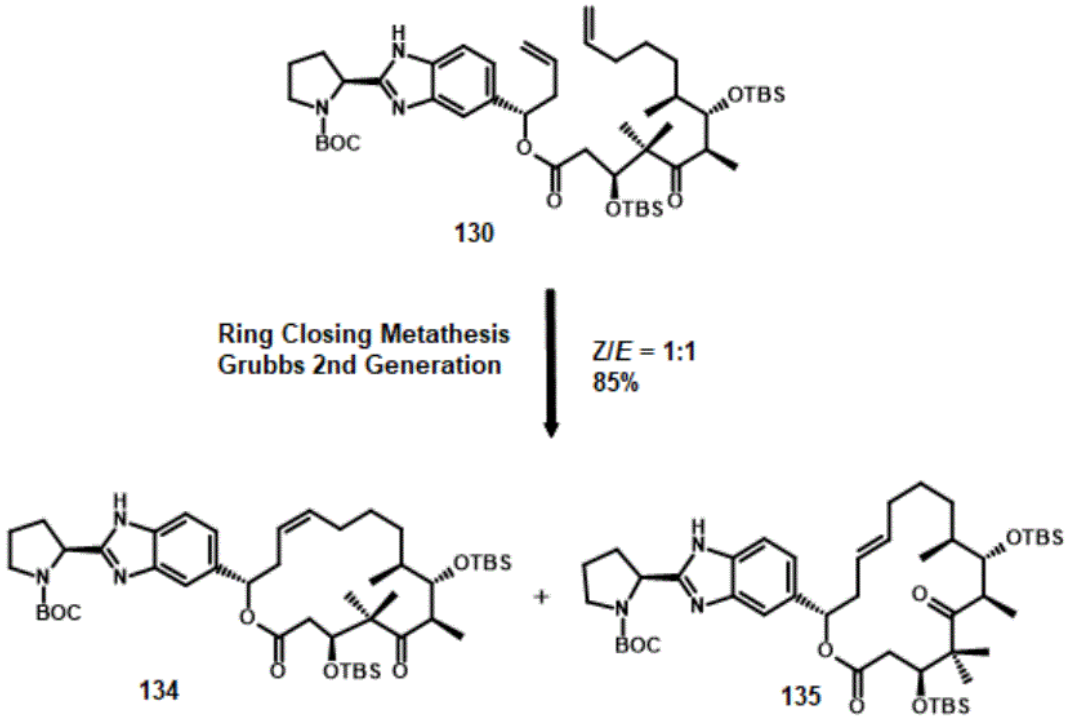


Figure 4.23: RCM by using Grubbs 2nd generation ruthenium catalyst

Finally, the ring-closing metathesis product containing a mixture of *Z* and *E* isomers (**134** and **135**) were deprotected with the reagents $(HF)_3.Et_3N$ and Et_3N in CH_3CN solvent by heating the reaction mixture in an oil bath at 45 ° C for 24 hours. The reaction proceeded very cleanly without any kind of decomposition in 70% yield.

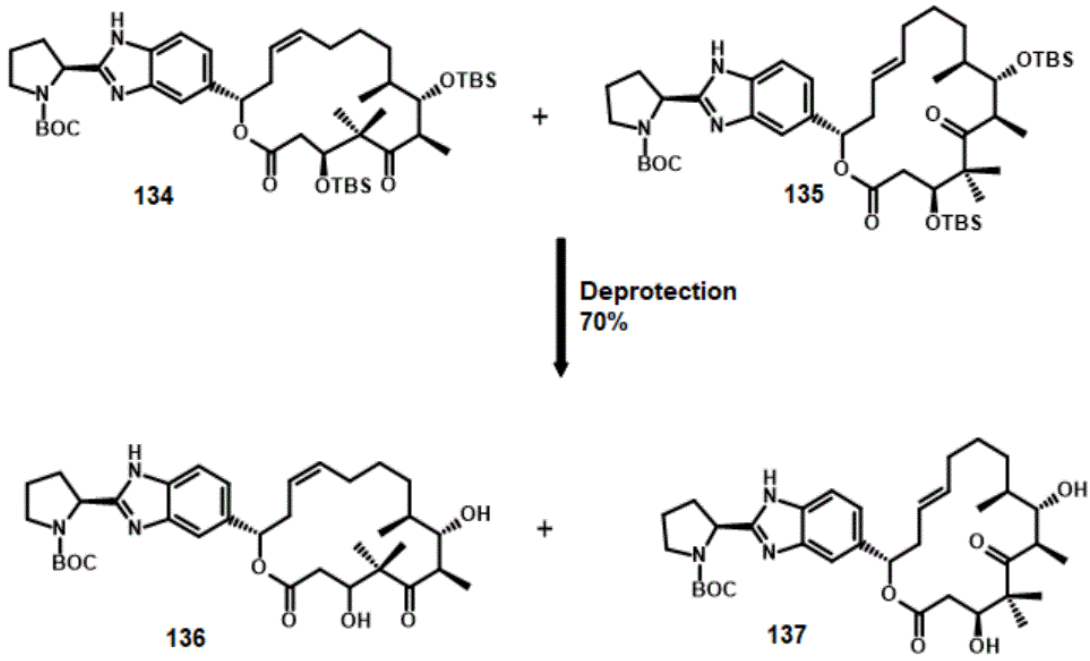


Figure 4.24: Epothilone having proline fused benzimidazole ring

4.8 Outlook and Future Prospective

Each year in the medicinal world, new chemical entities get licensed for medicinal use. But, the journey begins with the universities laboratories research where the researcher at molecular level tries to understand the pathways and processes behind the disease. After the identification of potential target, the researchers then get focused on the molecules in most cases are natural products that can best fit on the target and in that way facilitate to hinder the mechanism of propagation of disease. To get the molecule licensed in the market, risks to benefits ratio is the basic parameter, by which molecule not only to be proved effective but it should also be safe for the intended use. The modifications in the molecule such as Paclitaxel to Docetaxel had shifted the risks to benefits ratio dramatically by enhancing the efficacy and reducing the side effects.

The new synthesis strategy used to produce proline fused benzimidazole ring through dehydration of amides, proved to be very robust and provide an excellent synthesis route which can be used in the synthesis of new amino acid fused benzimidazole side chains of epothilone and consequently new analogues can be synthesized efficiently. The search of a molecule with, the improved

availability, increased efficacy, reduced side effects and the broader scope of clinical application in medicinal chemistry is the main reason to synthesize new analogues. So, the approach carried out in this work, in the future can be used to produce the new analogues of amino acid fused benzimidazole ring by which the promising preclinical data can be generated that might lead to discover a molecule providing an alternative to existing therapies in the market. It is recommended for future works in case of problem with the separation of *Z* and *E* isomers, specially designed silver nitrate instead of silica gel as stationary medium can be used in flash chromatography.

Chapter 5

Experimental Section

5.1 Analytical and Working Techniques

All required fine chemicals were purchased from the big fine chemical companies such as ACROS , ALDRICH , FLUKA and MERCK. Most of them were used directly without further purification if nothing else was mentioned. All solvents were distilled and dried before use. Anhydrous solvents were obtained as follows: THF, diethyl ether and toluene by distillation from sodium and benzophenone. Unless mentioned, all the reactions were carried out under a nitrogen atmosphere and the glass material was pre-dried by flame drying under high vacuum (oil pump RV 5, EDWARDS). All air or water sensitive chemicals were stored under inert atmosphere. Compounds synthesis mentioned in experimental section were prepared according to conditions mentioned in literatures.[54][51][64][60][61][62]

5.1.1 NMR-Spectroscopy

^{1}H , ^{13}C NMR and two-dimensional spectra (COSY, TOCSY, HSQC, HMBC, NOESY) were measured on BRUKER DPX 400, AMX 200, AMX 400 and BRUKER AMX 600 (600 or 150 MHz, respectively). As solvents was used chloroform−d or benzene−d6. TMS ($\delta = 0$) was used as an internal standard. Data are reported as follows: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet m =multiplet, br = broadened), coupling constant (Hz), integration, peak assignment]. For the ^{13}C NMR spectra the signal multiplicity is determined by means of the APT or DEPT-135 technique: d for CH, q for CH_3, t for CH_2, and S for C.

5.1.2 Mass Spectrometry

Mass spectra were recorded on a Finnigan SSQ 7000 from the FINNIGAN-MAT (Bremen). High-resolution mass spectra were measured on an Intectra Finnigan MAT-95 mass spectrometer from the same firm. The used mass spectrometric ionization methods were electron-impact (EI) with 70eV ionization potential, chemical ionization (CI) with NH3 as gas reactant, fast-atom bombardment (FAB) or fielddesorption (FD). Significant fragments are reported as follows: m/z (relative intensity).

5.1.3 Infrared Spectrometry

Infrared spectra (IR) were recorded on a FT-IR (fourier transform infrared spectroscopy) from the firm PERKIN ELMER. The percent transmittance (T%) of liquid or oily substances was measured in film between potassium bromide tablets. Solid substances were pulverized with potassium bromide and percent reflection (R%) was measured. Absorption band frequencies are reported in cm-1.

5.1.4 Polarimetry

Optical rotations were measured on a Perkin-Elmer Polarimeter P-341. They are reported as follows: $[\alpha]_D^{Temperature}$ (concentration, solvent). The unit of c is g/100 mL. As a solvent was used anhydrous CH_2Cl_2.

5.1.5 Melting Point

Melting points were taken with a BUECHI B-540 point microscope apparatus or digital Electrothermal IA 9100 from Kleinfeld company and were not corrected.

5.1.6 Elemental Analysis

Elemental analyses were recorded with a LECO CHNS-932.

5.1.7 Chromatographic Analysis

Analytical thin-layer chromatography (TLC) was performed on pre-coated silica gel $60F_{254}$ plates (MERCK) or POLYGRAM SIL G/UV_{254} (MACHEREY-NAGEL), and precoated aluminum oxide ALOX N/UV_{254} (MACHEREY-NAGEL). The compounds were visualized by UV_{254} light and the chromatography plates were

developed witha vanillin solution or aqueous solution of potassium permanganate (heating on a hotplate).

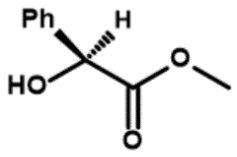
For preparation of the vanillin solution were used 8.6 g vanillin dissolved in 200 mL ethanol and put 2.5 mL H_2SO_4. The potassium permanganate solution was prepared from 2.5 g $KMnO_4$ and 12.5g Na_2CO_3 in 250 mL H_2O and 5 mL 5% NaOH.Flash column chromatography was performed using flash silica gel 60 M (40-63 μm)from the firm FLUKA.

5.2 Synthesis strategy

Total synthesis of proline fused benzimidazole ring epothilone was carried out first by the synthesis of its main fragments such as Ethyl-*S*-ketone **(93)**, Chiral aldehyde **(65)** and benzimidazole fragment **(126)**. The fragments Ethyl-*S*-ketone **(93)**, Chiral aldehyde **(65)** were coupled by using aldol reaction for building of epothilone ring and resulting aldol product **(106a)** further converted to acid **(110)** was lactonised with benzimidazole fragment **(126)**. At the end, the resulting epothilone metathesis substrate **130** was closed by using ring closing metathesis (RCM) and subsequently that products were deprotected to furnish proline-fused benzimidazole epothilone *Z* isomer **136** and *E* isomer **137**. The whole experimental procedures for total synthesis of epothilone analogue has been discussed in the following part of this chapter.

5.3 Synthesis of Ethyl-*S*-ketone (93)

5.3.1 *(S)*-(+)-Mendalmethylaceticester (82)



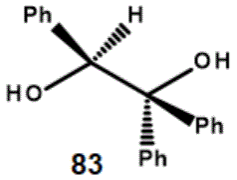
82

To the solution of mandelic acid **81** (20 g, 131.57 mmol) in 100 ml of absolute CH_3OH, concentrated H_2SO_4 (221 μL, 4.14 mmol, 0.03 eq) was added and then refluxed for 5 hours. The reaction was neutralized with K_2CO_3 (316.8 mg, 2.29 mmol) solution in 421 μL water and after that methanol was evaporated

by using high vacuum. After removing methanol, 19.7 g of product *(S)*-(+)-Mendalmethylaceticester **(82)** having 97% yield was recrystallized by using hexane as solvent.

General Data: $C_9H_{10}O_3$ FW:166.17; TLC: R_f=0.35(Pentane/Et_2O 1:1; UV$(+)$; Vanillin: yellow

5.3.2 *(S)*-(-)-1,1,2-Triphenyl-1,2-ethandiol (83)



83

Bromobenzene (1.8 mL, 17 mmol, 0.16 eq) was added dropwise to the stirring mixture of magnesium powder (15 g, 671 mmol, 6.05 eq) with Iodine in absolute Et_2O under inert condition. When the reaction mixture started to boil gently then the bromobenzene (62.50 mL, 590 mmol, 5.54 eq) was added dropwise within one hour. After stopping the addition, the reaction was allowed to run at room temperature for 4.5 hours. In the end, the reaction mixture cooled to 0 °C with ice, and then *(S)*-mandelicester **82** (18.5 g, 111 mmol, 1 eq) in 100 mL Et_2O and 10 mL THF cooled with ice was added dropwise within 90 min maintaining the temperature around about 10 °C. The reaction was allowed to run overnight at room temperature then was refluxed for one hour.

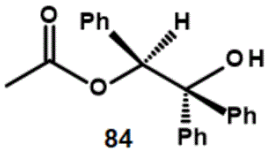
After letting the reaction mixture to cool down to room temperature, 200 g ice was added into it. Then the reaction mixture was neutralized by using the 5% HCl solution and 50 g ice. In the end, reaction mixture was allowed to run at room temperature for one hour. Then the organic phase was separated and washed with a saturated solution of $NaHCO_3$ and dried with $MgSO_4$. After evaporating the solvent, crude product was recrystallized with methanol. The whole process resulted to have product **83** (21.9 g, 75.5 mmol) with 70% yield as a white needle crystal.

General Data: $C_{20}H_{18}O_2$ FW:290.36, MP:50-55 °C, $[\alpha]_D^{20}$=−215.5(c =1.0, $CHCl_3$) TLC:R_f=0.25 (Pentane/Et_2O 2:1); UV$(+)$; Vanillin: yellow

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 7.61-7.65(m, 2H;H_{arom}), 7.00-7.40(m, 13H; H_{arom}), 5.54(d, ^3J=3.1 Hz, 1H;H-2), 3.19(s, 1H;Ph_2COH), 2.59(s, 1H;Ph-COH),

^{13}C-NMR(100 MHz, $CDCl_3$): 145.05, 143.31, 138.7(s, Ph-1), 128.36, 128.04(d, Ph-2, Ph-3), 127.61(d, Ph-4), 127.54, 127.37(d, Ph-2, Ph-3), 127.28, 126.24(d, Ph-4), 126.98, 126.12(d, Ph-2,Ph-3), 80.69(s, C-1), 76.68(d, C-2).

5.3.3 *(S)*-(-)-2-Hydroxy-1,2,2-triphenylethylacetate (84)



84

A solution of scandium (III) trifluoromethanesulfonate (0.702 g, 1.42 mmol) in anhydrous acetonitrile (ACN) was added dropwise to the stirred solution of the *(S)*-(-)-1,1,2 triphenyl-1,2-ethandiol **83** (20 g, 69.12 mmol) and acetic anhydride (9.77 mL, 0.103 mol) in the 500 mL anhydrous acetonitrile (286 mL) under the nitrogen atmosphere at 0 °C. The order of above mentioned reagent addition was very critical to have the optimum yield. The addition of catalyst should be carried out very slowly and continuously at a constant pace. After the addition of catalyst solution, the white precipitate (ppt) began to appear in the reaction solution and then the reaction was allowed to stir at room temperature for another 3 hours.

The solid ppt was filtered and washed with ACN (2 X 25 mL) and dried under vacuum at 40 °C overnight. The whole process resulted in the product *(S)*-(-)-2-hydroxy-1,2,2-triphenylethylacetate (*S*-HYTRA) **84** as the white solid (16.4 g, 71.4% yield).

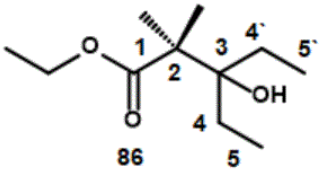
General Data: $C_{22}H_{20}O_3$ FW:332.39, MP:225-230 °C, TLC:R_f =0.40 (Pentane/Et_2O 2:1), UV($+$), Vanillin: yellow.

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 7.55-7.57(m, 2H;H_{arom}), 7.04-7.42(m, 13H; H_{arom}), 6.68(s, 1H;PhCH), 2.82(s, 1H;Ph_2COH), 1.98(s, 1H; CH_3CO_2-CH).

^{13}C-NMR(100 MHz, $CDCl_3$): 169.69(s, CH_3CO_2CH), 144.82, 142.65(s, Ph-1), 128.44, 128.35(d, Ph-2, Ph-3), 127.91(d, Ph-4), 127.77, 127.63(d, Ph-2, Ph-3), 127.45, 127.33(d, Ph-4), 126.99, 126.17(d, Ph-2, Ph-3), 80.29(s, Ph_2C), 78.50(d, PhCH), 21.11(q, CH_3CO_2CH)

IR(Film): $\widetilde{\nu}$(cm^{-1}): 3064(m), 3024(s), 1735(m), 1495(m), 1372(m), 1239(m), 1168(w), 779.

5.3.4 Ethyl 3-ethyl-3-hydroxy-2,2-dimethylpentanoate (86)



Firstly, the zinc suspension (20 g, 0.305 mol) in THF (75 mL) and $B(OMe)_3$ (74.14 mL) was activated by using the 1,2-dibromoethane (0.48 mL, 5.5 mmol) and TESOTf (0.63 mL, 2.7 mmol). Then a mixture of 3-pentanone (29.47 mL, 0.27 mmol) and ethyl 2-bromo-2-methylpropanoate (43.7 mL, 0.308 mol) was added slowly to the activated zinc suspension. The reaction mixture was heated gently in a hot air stream by using the heat gun until the reaction mixture started to boil. The reacting mixture was added at such a rate that the solvent gently started to boil. After the addition of reactants, mixture was refluxed for 2 hours and stirred at room temperature for 20 hours.

Then the reaction mixture was quenched by the addition of 25% aqueous NH_3 solution (83.4 mL) at 0 °C. Glycerine (83.4 mL) and Et_2O (74.1 mL) were added and the organic layer was separated by using the separating funnel. Then aqueous layer was extracted three times with Et_2O (50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The purification of residue was done by using vacuum distillation, which afforded β-hydroxyester **86** (37.3 g, 68 %) as a colorless liquid.

General Data: $C_{11}H_{22}O_3$, FW:209.29; BP:108-110 °C(10 mbar); TLC:R_f-=0.48 (Pentane/Et_2O 1:1). UV (−), Vanillin: dark-blue.

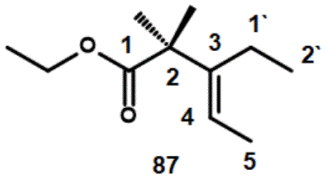
^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 4.17 (q, ^3J=7.1 Hz, 2 Hz; CH_3CH_2OCO), 3.78 (s, 1H; OH), 1.56 (m, 4H, H-4, H-4'), 1.29 (t, ^3J=7.1 Hz, 3H; CH_3CH_2OCO) 1.22 (s, 6H; C2-$(CH_3)_2$), 0.93 (t, ^3J=7.5 Hz, 6H; H-5, H-5')

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm): 179.06(s, C-1), 76.09(s, C-3), 60.82(t, CH_3CH_2OCO), 50.25(s, C-2), 28.08(t, C-4, C-4′), 21.52(q, C-5, C-5'), 13.98(q, CH_3CH_2OCO), 8.80(q, C2-$(CH_3)_3$).

IR(Film): $\tilde{\nu}$(cm^{-1}): 3492(m), 2982(s), 1699(s), 1472(s), 1390(m), 1271(s), 1153(S), 1026(m), 968(m), 857(w), 776(w)

MS(EI) : m/z(%): 203.4(100)[M^+ +H], 185.4(78)[M^+-H_2O], 171.1(11), 155.1 (23), 145.1(24), 111.1(16)

5.3.5 *(E)*-3-Ethyl-2,2-dimethyl-3-penenoicethylester (87)



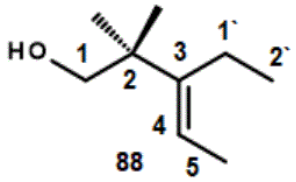
Hydroxy ester **86** (12.2 g, 60.41 mmol) was heated under reflux with Sicapent (14.83 g) in cyclohexane (52 mL) for 20 min. The solvent was removed by using the rotary evaporator. Then, Vacuum distillation of the residue resulted into ester **87** (9.2 g, 82.5 %) as a colorless liquid.

General Data: $C_{11}H_{20}O_2$, FW:184.28; BP:60-63 °C(10 mbar); TLC:R_f=0.75 (Pentane/Et_2O 2:1), UV (−), Vanillin: light-blue.

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.41 (q, ^3J=6.8 Hz, 1H; H-4), 4.11 (q, ^3J=7.2 Hz, 2Hz; CH_3CH_2OCO), 2.07 (q, ^3J=7.6 Hz, 2H; H-1'), 1.66 (d, ^3J=6.8 Hz, 3H; H-5), 1.28(s, 6H;C2-$(CH_3)_2$), 1.23(t, ^3J= 6.8 Hz, CH_3CH_2OCO), 0.97(t, ^3J=7.5 Hz, 3H;H-2')

^{13} C-NMR(100 MHz, $CDCl_3$): δ(ppm): 177.19 (s, C-1), 144.10(s, C-3), 118.49(d, C-4), 60.33(t, CH_3CH_2OCO), 48.47(s, C-2), 24.90(q, C2-$(CH_3)_3$), 21.68(t, C-1′), 14.09, 13.90, 13.54(q, C-5, CH_3CH_2OCO, C-1')

5.3.6 *(E)*-3-Ethyl-2,2-dimethyl-3-penten-1-ol (88)



LAH (3.30 g, 86.91 mmol, 4.0 eq) was added carefully to a solution of ester **87** (8.0 g, 43.4 mmol) in THF (47 mL). The mixture was refluxed for 2 hours. After cooling to 0 °C, Et_2O (30 mL) was added, and the mixture was quenched carefully by dropwise addition of water (3 mL), 15% aqueous NaOH (3 mL), and water (5 mL). The Celite (400 mg) was added and the mixture was stirred for 30 min at room temperature.

The precipitate was filtered off by suction and washed with Et_2O (4 x 40 mL). The filtrate and washings were combined and concentrated in vacuo to furnish crude *(E)*-3-ethyl-2,2-dimethyl-3-penten-1-ol **88** a colorless liquid, which was used for the preparation of aldehyde **89** without further purification. An analytical sample of compound **88** was obtained by using kugelrohr distillation.

General Data: $C_9H_{18}O$, FW:142.24; BP:105-106 °C(30 mbar); TLC:R_f=0.40 (Pentane/Et_2O 5:1), UV (−), Vanillin: light blue.

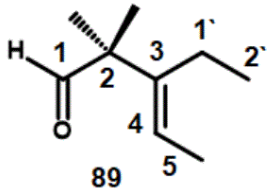
^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.40(q, ^3J= 6.7 Hz, 1H; H-4), 3.35(s, 2H;H-1), 2.08(q, ^3J=7.6 Hz, 2Hz;H-1'), 1.67 (d, ^3J= 6.7 Hz, 3H; H-5), 1.32(br s, 1H;OH), 1.05(s, 6H; C2-$(CH_3)_3$), 0.98(t, ^3J=7.5 Hz, 3H;H-2')

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm): 145.21(s, C-3), 120(d, C-4), 69.76(t, C-1), 41.99(s, C-2), 24.03(q, C2-$(CH_3)_3$), 19.99(t, C-1'), 14.23, 13.62(q, C-5, C-2').

IR(Film): $\widetilde{\nu}$(cm^{-1}): 3393(br s), 2970(s), 1702(w), 1476(m), 1377(m), 1046(m), 960(w), 822(m), 643(w).

MS(EI) : m/z(%): 142.1(7)[M^+], 125.1(48), 111.0(34), 96.2(14), 83.0(35), 71.1(37), 69.1(100), 57.0(42), 55.0(66).

5.3.7 *(E)*-3-Ethyl-2,2-dimethyl-3-pentenal (89)



89

DMSO (6.59 mL, 93.0 mmol, 2.0 eq) in CH_2Cl_2 (20 mL) was added carefully dropwise at -78 °C to a stirred solution of oxalyl chloride $(COCl)_2$ (3.71 mL, 42.7 mmol, 1.1 eq) in CH_2Cl_2 (97 mL) within 5 min. The mixture was stirred for 10 min at -78 ° C. The crude *(E)*-3-ethyl-2,2-dimethyl-3-penten-1-ol **88** dissolved in CH_2Cl_2 (40 mL) was added dropwise within 5 min. The mixture was then stirred for 1 h at -78 °C.

The reaction was quenched by dropwise addition of NEt_3 (27 mL, 194.0 mmol, 5.0 eq). The mixture was warmed to room temperature within 45 min. Water (97 mL) was added, and the mixture was stirred for 10 min. The organic layer was separated and the aqueous phase was extracted three times with CH_2Cl_2 (40 mL). The organic phase was evaporated and the crude product was distilled under high vacuum. Which afforded the product **89** as a colorless oil by having the 72% yield in two steps (4.24 g).

General Data: $C_9H_{16}O$ FW:140.23; BP:85-86 °C (28 mbar); TLC:R_f=0.75 (Pentane/Et_2O 5:1); UV (−), Vanillin: dark blue.

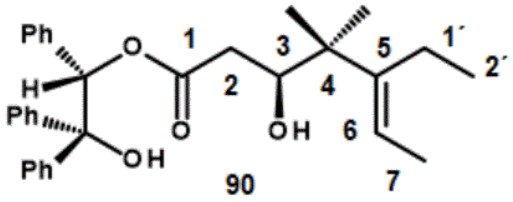
^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 9.27(s, 1H;H-1), 5.41(q, ^3J=6.8 Hz, 1H; H-4), 2.02(q, ^3J=7.6 Hz, 2H;H-1'), 1.69 (d, ^3J=6.9 Hz, 3H; H-5), 1.17(s, 6H; C2-$(CH_3)_3$), 0.98(t, ^3J=7.5 Hz, 3H;H-2')

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm): 203.40(s, C-1), 141.21(s, C-3), 122.58(d, C-4), 52.63(s, C-2), 20.90(t, C-1'), 14.06, 13.72(q, C-5, C-2')

IR(Film): $\widetilde{\nu}$(cm^{-1}): 3403(m), 2974(s), 1728(m), 1472(m), 1376(m), 1144(m), 1086(w), 8975(w), 825(w).

MS(EI) **:** m/z(%): 141.0(30)[M^+H], 127.1(98), 111.0(100), 97.1(2), 83.0(3).

5.3.8 (3*S*,5*E*)-5-Ethyl-3-hydroxy-4, 4-di -methyl-5-heptanoicacid-(1*S*)-2-hydroxy-1,2,2-triphenylethylester (90)



To the solution of diisopropylamine(2.56 mL, 16.0 mmol) in 20 mL abs THF at -78 °C was added carefully dropwise *n*-BuLi solution (6.40 mL, 16 mmol, 2.5 molar solution in hexane) cooled at -78 °C under continuous stirring and inert atmosphere. After the addition, the reaction mixture was allowed to stir at 0 °C for 30 minutes. This freshly prepared LDA solution was added drop-wise to already cooled solution of*(S)*-HYTRA **84** (2.66 g, 8 mmol) in 60 mL abs THF at -78 °C. The deprotonation reaction was allowed to stir at 0 °C for 60 min. The resulting orange coloured solution of the *(S)*-HYTRA enolate was again cooled to -78 °C and then finally the aldehyde **89** (1.34g, 9.6 mmol) was added to it and the reaction mixture was allowed to stir for 2.5 hours at -78 °C.

In the end, reaction was quenched with saturated solution of NH_4Cl (60 mL), the organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3 X 100 mL). The combined organic phase was dried over the anhydrous $MgSO_4$ and then evaporated to get the crude product. The crude product was purified by using flash chromatography (Pentane/Et_2O $3{:}1$). The whole process resulted into the β-hydroxyester **90** (2.87 g, 76%, 94$\%de$) as colorless crystalline solid.

General Data: $C_{31}H_{36}O_4$ FW:472.62, MP:144-145 °C, $[\alpha]_D^{20}$=−163.3(c =1.0, $CHCl_3$), TLC:R_f=0.40(Pentane/Et_2O 2:1), UV$(+)$, Vanillin: green

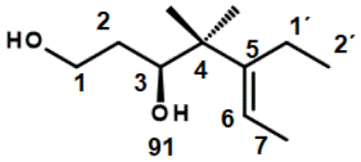
^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 7.59-7.54(m, 2H;H_{arom}), 7.38-7.02(m, 13H; H_{arom}), 6.70(s, 1H;PhCH), 5.30(q, ^3J=6.8 Hz, 1H;H-3), 3.78(ddd, ^3J=10.0 Hz,^3J=2.7 Hz,^3J=2.5 Hz, 1H;H-3), 2.87(s, Ph_2COH), 2.31(dd, ^2J=15.7 Hz, ^3J=2.2 Hz, 1H;H-2), 2.21(d, ^2J=15.7 Hz, ^3J=2.2 Hz, 1H;H-2), 2.03(d, ^3J=3.1 Hz, 1H;C3-OH), 1.98(dq, ^4J=2.2 Hz, ^3J=7.5 Hz, 2H;H-1'), 1.60(d, ^3J=6.8 Hz, 3H;H-2), 0.98(s, 3H;C4-CH_3), 0.91(s, 3H;C4-CH_3), 0.91(d, ^3J=6.8 Hz, 3H;H-2')

^{13}C-NMR(100 MHz, $CDCl_3$): 172.17(s, C-1), 145.95, 144.69(s, Ph-1), 142.58(s, C-5), 135.56(s, Ph-1), 128.38, 128.27(d, Ph-2, Ph-3), 127.93(d, Ph-4), 127.79, 127.48(d, Ph-2, Ph-3), 127.28, 127.06(d, Ph-4), 126.31, 126.27(d, Ph-2, Ph-3), 120.32(d, C-6), 80.32(s, Ph_2C), 78.91(d, PhCH), 72.30(d, C-3), 43.95(s, C-4), 37.33(t, C-2), 22.88(q, C4-CH_3), 21.27(q, C4-CH_3), 20.22(t, C-1'), 14.22, 13.60(q, C-7,C-2').

IR(Film): $\widetilde{\nu}$(cm^{-1}): 3548(m), 3465(m), 2972(m), 1724(s), 1494(w), 1450(m), 1290(m), 1153(s), 990(m), 890(w), 751(m), 699(s)

MS(EI) **:** m/z(%): 472.2(<0.4)[M^+], 455.2(0.4), 290.3(4), 273.1(70), 256.1(12), 195.1(17), 183(100), 167.1(12), 112.0(16), 105.0(26), 69.2(10).

5.3.9 (3*S*,5*E*)-5-Ethyl-4,4-dimethyl-5-hepten-1,3-diol (91)



LAH (1.30g, 34.3 mmol, 7.0 eq) was added portion-wise to refluxing solution of ester **90** (2.31 g, 4.88 mmol) in Et_2O (50 mL) within a period of 2 h. Refluxing was continued for another 30 min. After cooling to 0 °C, the reaction was quenched by drop-wise addition of water (1.35 mL) and 15 % aqueous NaOH (1.35 mL) and then Et_2O (40 mL) and water (1.35 mL) were added.

The mixture was stirred for 1 h at room temperature until a white precipitate formed, which was filtered off by suction through a small plug of celite. The precipitate was washed with Et_2O (4 x 40 mL). The filtrate and washings were combined and concentrated in vacuo. Purification of the residue by flash chromatography (pentane/Et_2O 2:1) resulted diol **91** (818 mg, 90%) as a colorless oil and (478 mg, 90%) as a colorless crystalline solid.

General Data: $C_{11}H_{22}O_2$ FW:186.29, $[\alpha]_D^{20}$=−30.3(c=1.0, $CHCl_3$), TLC:R_f =0.30 (Pentane/Et_2O 2:1), UV(−), Vanillin: dark blue

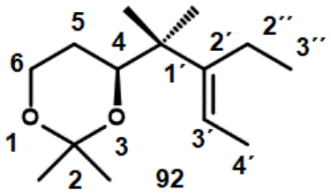
^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.45(q, ^3J = 6.8 Hz, 1H;H-6), 3.89-3.76(m, 2H;H-1), 3.70(dd, ^3J = 10.3 Hz,^3J = 2.2 Hz, 1H;H-3), 3.13(br, s, 1H;OH), 2.35(br, s, 1H; OH), 2.20-2.02(m, 2H; H-1'), 1.67(d, ^3J = 6.8 Hz, 3H;H-7), 1.65-1.01(m, 2H, H-2), 1.03(s, 3H;C4-CH_3) 1.01(s, 3H;C4-CH_3), 0.98(d, ^3J = 7.6 Hz, 3H;H-2')

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm): 145.52(s, C-5), 122.23(d, C-6), 77.30(d, C-3), 63.75(s, C-1), 45.69(t, C-4), 33.74(t, C-2), 23.90, 22.50 (q, C4-CH_3) 21.44(t, C-1'), 15.58, 14.91(q, C-7,C-2').

IR(Film): $\widetilde{\nu}$(cm^{-1}): 3313(br s), 2969(s), 1472(s), 1382(m), 1312(m), 1053(s), 956(m), 822(w), 659(w).

MS(EI) **:** m/z(%): 186.0(<0.6)[M$^+$], 177.0(1), 141.0(3), 112.1(100), 96.9(19), 83.0(75), 74.9(13), 68.9(60), 54.9(34).

5.3.10 *(S)*-4-(*E*-2-Ethyl-1',1'-dimethyl-2- butenyl)-2,2-dimethyl-[1,3]-dioxan (92)



Anhydrous $CuSO_4$ (600 mg, 3.76 mmol, 1.5 eq), p-TsOH. H_2O (95.36 mg, 0.50 mmol, 0.2 equiv), and anhydrous pyridine (30.12 mL, 0.376 mmol, 0.15 eq) was added to a solution of diol **91** (771 mg, 4.14 mmol) in anhydrous acetone (37.6 mL). The mixture was stirred for 24 h at room temperature.

Saturated aqueous $NaHCO_3$ solution (50.2 mL) was added and the aqueous layer was extracted with Et_2O (4 x 76 mL). The combined organic extracts were dried over $MgSO_4$ and carefully concentrated in vacuo. Purification of the residue by flash chromatography (pentane/Et_2O 40:1) gave the acetonide **92** (1.042g, 88%) as a colorless oil.

General Data: $C_{14}H_{22}O_2$ FW:226.29, $[\alpha]_D^{20} = +14.3$(c=1.0, $CHCl_3$), TLC:R_f =0.30(Pentane/Et_2O 2:1), UV$(-)$, Vanillin: darkblue

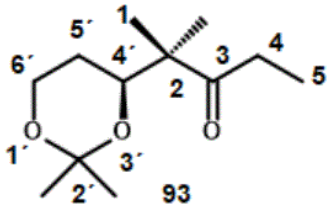
^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.33(q, ^{3}J=6.8 Hz, 1H;H-3'), 3.88(dt, ^{2}J=11.8 Hz, ^{3}J=2.9 Hz, 1H;H-6), 3.81(ddd, ^{2}J=11.6 Hz, ^{3}J=5.5 Hz, ^{3}J=2.0 Hz 1H;H-6), 3.72(dd, ^{3}J=11.6 Hz, ^{3}J=2.5 Hz, 1H;H-4), 2.15-2.00(m, 2H;H-1"), 1.63(d, ^{3}J=6.8 Hz, 3H;H-4'), 1.59-1.46(m, 1H;H-5), 1.41(s, 3H;C2-CH_3), 1.35(s, 3H;C2-CH_3), 1.18(ddd, ^{2}J=13.1 Hz, ^{3}J=4.7 Hz, ^{3}J=2.6 Hz 1H;H-5), 1.03(s, 3H;C1'-CH_3), 1.00(s, 3H;C1'-CH_3), 0.98(t, ^{3}J=7.5 Hz, 1H;H-2")

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm): 146.12(d, C-3'), 119.21(d, C-4'), 98.23(s, C-2), 60.35(s, C-6), 42.87(s, C-1'), 29.84(q, C2-(CH_3)), 26.14(t, C-5), 24.19, 21.14, 19.11(q, C2-CH_3, C1'-CH_3), 20.74(t, C-1"), 14.36, 13.59(q, C-4, C-2").

IR(Film): $\widetilde{\nu}$(cm^{-1}): 2970(s), 1475(s), 1380(s), 1271(m), 1197(s), 1108(m), 971(m), 860(m), 765(w).

MS(EI) **:** m/z(%): 226.2(8)[M^+], 211.1(14), 205.1(3), 151.1(14), 114.9(100), 94.7(10), 72.9(32), 58.9(60).

5.3.11 *(S)*-2-(2,2-Dimethyl-[1,3]-dioxan-4-yl-2-methylpentan-3-on (93)



A stream of ozone from ozonizer was bubbled through a solution of acetonide **92** (800 mg, 3.53 mmol) in CH_2Cl_2 (120 mL) at -78 °C until the blue colour of the solution persisted. PPh_3 (927 mg, 1.2 eq) was added at -78 ° C and then the mixture was allowed to warm to room temperature within 4 hours and concentrated in vacuum. The purification of the residue by flash chromatography (pentane/Et_2O 5:1) furnished the ethyl-*S*-ketone **93** (629 mg, 83%) as a colorless oil.

General Data: $C_{12}H_{22}O_3$ FW:214.30, MP: 37 °C; $[\alpha]_D^{20} = +12.6$(c=1.0, $CHCl_3$), TLC:R_f=0.30(Pentane/Et_2O 2:1), UV$(-)$, Vanillin: black

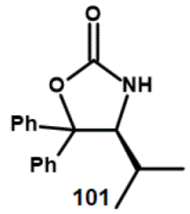
^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 4.06(dt, ^3J=11.7 Hz, ^3J=2.5 Hz, 1H;H-6), 3.96(dt, ^2J=11.8 Hz, ^3J=2.9 Hz 1H;H-6'), 3.87(ddd, ^2J=11.6 Hz, ^3J=5.5 Hz, ^3J=2.0 Hz, 1H;H-6'), 2.50(q, ^3J=6.8 Hz, 2H;H-4), 1.67-1.57(m, 1H;H-5'), 1.41(s, 3H;C2'-CH_3), 1.34-1.25(m, 1H;H-5'), 1.33(s, 3H;C2'-CH_3), 1.13(s, 3H;C-1), 1.07(s, 3H;C1'-CH_3), 1.01(t, ^3J=7.2 Hz, 3H;H-5)

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm): 215.54(d, C-3), 98.33(d, C-2'), 73.31(d, C-4'), 59.98(s, C-6'), 50.55(s, C-2), 31.69(t, C-4), 29.72(q, C2'-CH_3), 25.26(t, C-5'), 19.14, 18.95(q, C-1 ,C-2-CH_3, C-2'-CH_3), 7.86(q, C-5)

5.4 Synthesis of chiral-aldehyde 65

The synthesis of chiral-aldehyde **65** was carried out by using the synthesis of substances mentioned below:

5.4.1 *(S)*-4-Isopropyl-5,5-diphenyl-oxazolidin-2-one (101)



To the solution of 2-amino-1,1-diphenyl-butan-1-ol (29.3g, 114.7mmol) and NEt_3 (16.70 mL,126.15 mmol) in 555 mL absolute dry CH_2Cl_2 at -25 °C, ethylchloroformate $ClCO_2Et$ (8.87 mL, 114.7mmol, 1 eq) was added dropwise by continuous stirring. Then the reaction mixture was allowed to warm-up to room temperature and then stir it overnight. Then the reaction mixture was diluted with 255 mL CH_2Cl_2, then washed with 632 mL 1 N HCl, and then the organic phase was separated.

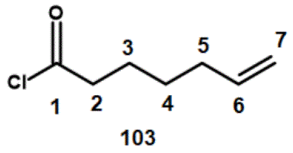
The solvent of reaction mixture was evaporated by using the rotary then solid precipitate was suspended in 632 mL 1 N NaOH solution in methanol. Then the suspension were refluxed for 9 hours and was diluted with water and cooled with ice bath then the suspension was filtered and washed with water, Et_2O (1 mL/mmol) and then with pentan. The purification was ended by doing the recrystallization with ethyl acetate which resulted in the 23.32g (72%) oxazolidine **101** as white crystalline solid.

General Data: $C_{18}H_{19}NO_2$ FW:281.35, MP: 251-252 °C; $[\alpha]_D^{20} = -260$ (c=1.0, $CHCl_3$), TLC:R_f=0.30(Pentane/Et_2O 2:1), UV$(+)$, Vanillin: $(-)$

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 7.46-7.49(m, 2H;H_{arom}), 7.18-7.33(m, 8H; H_{arom}), 6.14(s, 1H;H-3), 4.29(d, ^3J=3.6 Hz, 1H;H-4), 1.76-1.85(m, 1H; $(CH_3)_2CH$), 0.83(d, ^3J=6.9 Hz, 3H;$(CH_3)_2CH$), 0.62(t, ^3J=6.3 Hz, 3H;$(CH_3)_2$-CH).

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm): 158.50(s, C-2), 143.88, 139.16(s, Ph-1), 128.19, 128.16, 128.06, 127.68(d, Ph-2), 126.32, 125.70(d, Ph-3), 89.35(t, C-5), 65.81(d, C-4), 29.58(d, $(CH_3)_2CH$), 20.84, 15.60(q, $(CH_3)_2CH$).

5.4.2 Hept-6-enoylchloride (103)

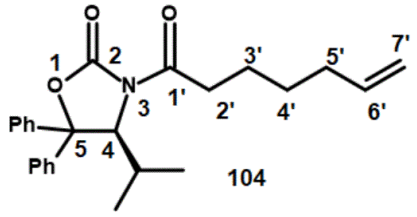


103

To the solution of 2.4 mL (2.25 g, 17.55 mmol) of 6-heptenoic acid **102** in 9 mL abs CH_2Cl_2 was added 3 mL (4.47 g, 35.2 mmol, 2eq) oxalyl chloride. The reaction mixture was allowed to stir for 1 hr at room temperature and then at 40 °C for 1 hr. Then the reaction was allowed to cool, after the evaporation of solvent, furnished 2.54 g (100%) of acid chloride **103** as colorless oil.

General Data: $C_7H_{11}ClO$, FW:146.62, TLC:R_f=0.57 (Pentane/Et_2O 1:1), UV($-$), Vanillin: black

5.4.3 *(S)*-3-Hept-6-enoyl-4-isoprpyl-5,5-diphenyl- oxazolidin-2-on (104)



104

To the solution of 4-isopropyl-5,5-diphenyl-oxazolidin-2-one (900 mg, 3.67mmol, 1 eq) in dry 10 mL THF at 0 °C under strong stirring was added the solution of *n*-BuLi 2.33 mL (3.501 mmol, 1.15 eq, 1.6 M solution in hexane) dropwise. Then the 586.6 mg of acid chloride **103** in dry 2.7 mL THF was added dropwise at 0 °C under continuous stirring. The reaction mixture was allowed to stir at room temperature for 20 hours and then quenched with saturated $NaHCO_3$ solution.

The reaction mixture was allowed to stir for 1hour at 0 °C. The organic phase was separated and then the water phase was extracted three times with 50

mL Et_2O. Then the combined organic phases were first dried with $MgSO_4$ and then evaporated by using the evaporation rotary. The crude product was purified by using flash chromatography (Pentane/Et_2O 5:1). The 1.19 g of N-acyloxazolidinon **104** as colourless product (92% yield) was obtained.

General Data: $C_{25}H_{29}O_3$, FW: 391.51, $[\alpha]_D^{20} = -42$(c=1.0, $CHCl_3$), TLC:R_f =0.50 (Pentane/Et_2O 3:1), UV$(+)$, Vanillin: black

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 7.46-7.49(m, 2H;H_{arom}), 5.83-5.67(m, 1H;H-6'), 5.38(d, ^3J=3.4 Hz, 1H;H-4), 4.99-4.89(m, 2H;H-7'), 2.92-2.72(m, 2H;H-2'), 2.10-1.93(m, 3H;H-5',$(CH_3)_2CH$), 1.67-1.50(m, 2H;H-3'), 1.38-1.27(m, 1H;H-4'), 0.88(d, ^3J=7.0 Hz, 3H;$(CH_3)_2CH$), 0.77(d, ^3J=6.8 Hz, 3H;$(CH_3)_2CH$)

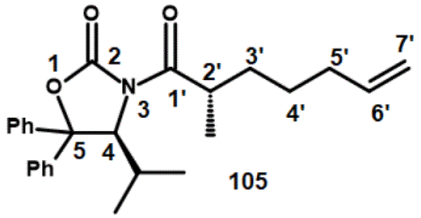
^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm): 173.04(s,C-1'), 153.02(s, C-2), 142.32(d, C-6') 142.32, 138.37(s, Ph-), 2 x 128.84, 2 x 128.30 , 127.90, 2 x 125.86(d, Ph-2), 2 x 125.56(d, Ph-1), 114.58(t, C-7'), 89.30(s, C-5), 64.42(d, C-4), 34.94(t, C-2'), 33.35(d, C-5'), 29.81(d, $(CH_3)_2CH$), 28.81(t, C-3'), 24.04(t, C-4'), 20.75, 16.36(q, $(CH_3)_2CH$)

IR(Film): $\widetilde{\nu}$(cm^{-1}) :3064(w), 2966(m), 2932(m), 2877(w), 1786(s), 1706(s), 1639(w), 1494(w), 1450(m), 1364(s), 1319(m), 1286(w), 1246(m), 1210(s), 1176(s), 1122(w), 1094(w), 1050(m), 991(w), 761(m), 704(m), 666(w)

MS(EI) : m/z(%): 391(10, [M^+]), 348(9), 323(4, [M^+]-C_5H_10), 281(3, [M^+]-C_7H_11 O), 263(4), 239(8), 238(24), 220(30), 194(31), 183(100), 165(27), 152(8), 128(4), 111(21), 105(16), 83(12), 77(8), 55(15)

HRMS(EI): calculated : 391.2147
found : 391.2148

5.4.4 4*(S)*-Isopropyl-3-(2-methylhept-6-enoyl) 5,5-diphenyl-oxazolidin-2-on (105)



To the solution of N-acyloxazolidinons **104** (1.10 g, 2.38 mmol) in 15 mL dry THF at -78 °C was added 2.78 mL of NaHDMS solution (2.73 mmol, 1.15 eq, 1.0 M in THF) drop-wise. After the addition, the reaction was allowed to stir at -78 °C for 1 hour, then MeI (0.87 mL, 13.92 mmol, 5 eq) was added dropwise at -78 °C and stirred for 4 hours at the -78 °C and then allowed to stir at room temperature for 20 hours.

The reaction was quenched with saturated solution of NH_4Cl, the organic phase was separated and the water phase was three times extracted with Et_2O. The combined organic phase was dried with $MgSO_4$ and then evaporated by using the rotary. Then 920 mg of methylated N-acyloxazolidinon product **105** was purified by using flash chromatography ((Pentane/Et_2O 10:1) by having the 79% yield.

General Data: $C_{26}H_{31}NO_3$, FW:405.23, $[\alpha]_D^{20} = -140$(c=1.0, $CHCl_3$), TLC: R_f =0.51(Pentane/Et_2O 3:1), UV$(+)$, Vanillin: violet

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 7.50-7.24(m, 10H;H_{arom}), 5.63-5.53(m, 1H;H-6'), 5.38(d, ^3J=3.4 Hz, 1H;H-4), 3.64-3.45(m, 1H;H-2'), 2.08-1.93(m, 3H;H-5', C2'-CH_3), 1.82-1.72(m, 2H;H-3'), 1.51-1.37(m, 2H;H-3'), 1.38-1.27(m, 1H;H-4'), 0.87(d, ^3J=7.0 Hz, 3H;$(CH_3)_2CH$), 0.78(d, $^3J = 6.7$ Hz, 3H;$(CH_3)_2CH$)

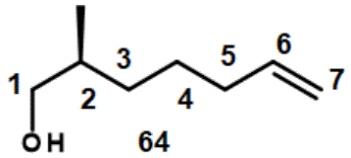
^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm): 176.92(s,C-1'), 152.80(s, C-2), 142.32(d, C-6') 138.35, 138.01(s, Ph-1), 2 x 128.79, 128.53, 2 x 128.36, 127.89, 2 x 125.81(d, Ph-2), 2 x 125.52(d, Ph-3), 114.41(t, C-7'), 89.24(s, C-5), 64.62(d, C-4), 37.10(t, C-2'), 33.47(t, C-5') 32.75(d, $CH(CH_3)_2$), 29.70(t, C-3'), 25.83(t, C-4'), 21.66, 17.93(q, $CH(CH_3)_2$, C2'-CH_3) 16.36(q, $(CH_3)_2CH$)

IR(Film): $\widetilde{\nu}$(cm^{-1}) : 3064(w), 2968(m), 2934(s), 2877(w), 1785(s), 1701(s), 1450(s), 1385(m), 1316(m), 1228(m), 1176(s), 1095(w), 912(w), 761(s), 748(m)

MS(EI) : m/z(%): 405(9, [M^+]), 362(8), 327(24), 318(4), 293(4), 263(8), 238(16), 220(40), 194(27), 183(100), 165(24), 158(8), 125(32), 77(8), 55(24).

HRMS(EI): calculated : 405.2304
found : 405.2306

5.4.5 2-*(S)*-Methylhept-6-en-1-ol (64)



To the solution of methylated N-acyloxazolidinon **105** (870 mg, 2.14 mmol) in dry 15 mL Et_2O at 0 °C was added portion wise LAH (253 mg, 6.65mmol, 3 eq) under strong stirring. The reaction mixture was refluxed for 2 hours and then quenched with water(10 mL) at 0 °C very cautiously. Then the quenched reaction mixture was filtered over Celite pad and the solid sediments were washed with Et_2O several times and the 283 mg (99%) product **64** was carefully collected by using the vacuum.

General Data: $C_8H_{16}O$, FW:128.21, $[\alpha]_D^{20} = -11.5$(c=0.5, $CHCl_3$), TLC :R_f =0.50(Pentane/Et_2O 3:1), UV($-$), Vanillin: deep blue

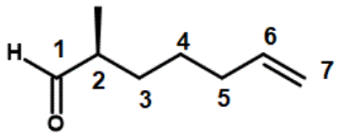
^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.86-5.76(m, 1H;H-6), 5.03-4.93(m, 2H;H-7), =3.4 Hz, 1H;H-4), 5.03-4.93(m, 1H;H-7), 3.52-3.40(m, 2H;H-1), 2.32(s, 1H;OH), 2.10-2.03(m, 2H;H-5), 1.65-1.33(m, 5H;H-2, H-3, H-4), 0.93(d, ^3J=6.7 Hz, 3H;C2-(CH_3))

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm): 138.89(d, C-6), 114.35(t, C-7), 68.23(t, C-1), 35.61(d, C-2), 33.99(t, C-5), 32.56(t, C-3), 26.25(t, C-4), 16.50(q, C2-(CH_3))

IR(Film): $\widetilde{\nu}$(cm^{-1}) : 3351(s), 3077(s), 2929(s), 2874(s), 1642(m), 1463(m), 1380(w), 1035(s), 910(s).

MS(EI) **:** m/z(%): 405(9, [M^+]), 362(8), 327(24), 318(4), 293(4), 263(8), 238(16), 220(40), 194(27), 183(100), 165(24), 158(8), 125(32), 77(8), 55(24)

5.4.6 2-(*S*)-Methylhept-6-enal (65)



65

To the solution of alcohol **64** (280 mg, 2.20 mmol) in 20 mL of dry CH_2Cl_2 at 0 °C was added drop-wise Dess-Martin-Periodinan (8.12 g, 2.82 mmol, 1.3 eq, 15% solution in CH_2Cl_2). The reaction mixture was allowed to stir at room temperature for 30 minutes. Then the reaction mixture was quenched with saturated solution of $Na_2S_2O_3$ and then $NaHCO_3$ solution. Then reaction mixture was filtered over the celite pad and then the sedimented product was washed several times with CH_2Cl_2. Then the organic phase was evaporated cautiously under the vacuum and 272 mg (97%) of chiral aldehyde **65** was obtained as colourless oil.

General Data: $C_8H_{14}O$, FW:126.21, $[\alpha]_D^{20} = +23.5$(c=1.0, $CHCl_3$), TLC:R_f =0.22(Pentane/Et_2O 7:1), UV($-$), Vanillin: deep blue

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 9.62(d, ^3J=2.0 Hz, 1H;H-1), 5.84-5.74(m, 1H;H-6), 5.84-5.74(m, 1H;H-6), 5.04-4.95(m, 2H;H-7), 2.38-2.32(m, 1H;H-2), 2.10-2.05(m, 2H;H-5), 1.77-1.33(m, 4H;H-3, H-4), 1.11(d, ^3J=7.0 Hz, 3H;C2-(CH_3))

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm): 205.18(d, C-1), 138.22(d, C-6), 114.89(t, C-7), 46.18(d, C-2), 33.64(t, C-5), 29.87(t, C-3), 26.14(t, C-4), 13.31(q, C2-(CH_3))

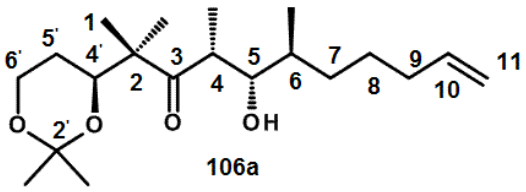
IR(Film): $\widetilde{\nu}$(cm^{-1}) **:** 3078(w), 2967(m), 2935(s), 2861(m), 2711(m), 1728(s), 1643(m), 1462(m), 912(s).

MS(EI) **:** m/z(%): 126(3,[M^+]), 125(8), 114(4), 111(8, [M^+]-H_2O), 97(17, [M^+-CHO]) 95(15), 82(13), 74(12), 71(32), 69(62), 67(24), 55(100), 54(23)

HRMS(EI): calculated : 128.1201
experimental : 128.1201

5.5 Aldol coupling between Chiral aldehyde 65 and Ethyl-*S*-Ketone 93

5.5.1 (4*R*,5*S*,6*R*,4'*S*)-2-(2,2-Dimethyl-[1,3]di- oxan-4-yl)-5-hydroxy-2,4-6-trimethylundec-10-en-3- on and (4*R*,5*R*-6*R*,4'*S*)-2-(2,2-dimethyl-[1,3]dioxan-4-yl)- 5-hydroxy-2-4-6-trimethyl-10-undecen-3-on (106a)



106a

A solution of ethyl-*S*-ketone **93** (0.525 g, 2.44 mmol) in dry THF (4.7 mL) was added to a freshly prepared solution of LDA [(*n*-BuLi (1.45 mL, 1.6 m solution in hexanes, 2.32 mmol, 0.96 eq) was added to a solution of diisopropylamine (0.339 mL, 2.42 mmol) in THF (11.0 mL) at 0 °C)] dropwise at -78 °C. The solution was stirred for 1 hour at - 78 °C. Then chiral aldehyde **65** (271 mg, 2.45 mmol, 1.0 equiv) was added dropwise and stirring was continued for 45 min at -78 °C.

The quenching of reaction mixture was carried out by dropwise addition of saturated aqueous NH_4Cl solution at -78 °C. The organic layer was separated and the aqueous layer was extracted with Et_2O. The combined extracts were dried over $MgSO_4$ and concentrated in vacuo.Flash chromatography (pentane/Et_2O 10:1) of the residue afforded major diastereomer **106a** (556 mg, 67%) and minor diastereomer **106b** (46.3 mg) as colorless oils (selectivity 12:1).

General Data: $C_{20}H_{36}O_4$, FW:340.50, $[\alpha]_D^{20} = -24.5$(c=1.0, $CHCl_3$), TLC :R_f =0.28(Pentane/Et_2O 3:1), UV($-$), Vanillin: black

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.84-5.74(m, 1H;H-10), 5.02-4.92(m, 2H;H-11), 4.02(dd, ^3J=11.8 Hz, ^3J=2.5 Hz, 1H;H-4'), 3.95(dt, ^2J=11.9 Hz,

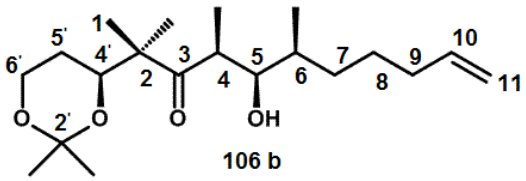
^3J=2.7 Hz, 1H;H-6'), 3.86(ddd, ^2J=11.7 Hz, ^3J=5.4 Hz, ^3J=1.7 Hz 1H;H-6'), 3.69(s, 1H;OH), 3.49(d, ^3J=7.0 Hz, 1H;H-5), 3.26(dq, ^3J=6.9 Hz, ^3J=1.4 Hz, 1H;H-4), 2.10-1.98(m, 2H;H-9), 1.83-1.70(m, 1H;H-7), 1.62-1.42(m, 3H; H-5', H-6, H-8), 1.41 (s, 3H, C2'-(CH_3)), 1.30, 1.31 (2s,2 x 3H, C2'-(CH_3)), 1.35-1.22(m, 2H; H-5', H-8), 1.18 (s, 3H;H-1), 1.15-1.05(m, 1H; H-7'), 1.07(s, 3H; C2-(CH_3)), 1.00(d, ^3J=7.0 Hz, 3H;C4-(CH_3)), 0.81(d, ^3J=6.8 Hz, 3H;C6-(CH_3)).

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm):222.20 (s, C-3), 138 (d, C-10), 114.77 (t, C-11), 98.468 (s, C-2'), 74.73 (d-C-5), 59.73 (t, C-6'), 51.34 (s, C-2), 45.5 (d, C-4), 35.31 (t, C-6), 34.2 (t, C-9), 31.7 (t, C-7), 29.71 (q, C2'-CH_3), 25.03 (t, C-5'), 21.24 (q, C-1), 19.00 (q, C2-CH_3), 18.05 (q, C2-CH_3), 15.32 (q, C6-CH_3), 9.28 (q, C4-CH_3).

IR(Film): $\tilde{\nu}$(cm^{-1}) **:** 3503(br,m), 3076(m), 2926(s), 2855(m), 1685(m), 1459 (m), 1379(m), 1272(w), 1197(m). 1161(m), 1106(s), 1055(m), 990(s), 971(s), 909(m)

MS(EI) : m/z(%): 340(<1, [M^+]) , 325(5, [M^+-CH_3]), 307(3), 264(11), 243(4), 226(4), 199(5), 185(15), 156(72), 137(12), 127(15), 115(81), 109(64), 99(25), 82(100), 69(67), 57(44).

HRMS(EI): calculated : 340.2614
found : 340.2615



106 b

General Data: $C_{20}H_{36}O_4$, FW:340.5, $[\alpha]_D^{20} = +12.5$(c=1.0, $CHCl_3$), TLC :R_f =0.17 (Pentane/Et_2O 3:1), UV($-$), Vanillin**:** black

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.84-5.74(m, 1H;H-10), 5.04-4.93(m, 2H;H-11), 4.04(dd, ^3J=11.8 Hz, ^3J=2.5 Hz, 1H;H-4'), 3.94(dt, ^2J=11.9 Hz, ^3J=2.7 Hz, 1H;H-6'), 3.84(ddd, ^2J=11.7 Hz, ^3J=5.4 Hz, ^3J=1.8 Hz 1H;H-6'), 3.45(dt, ^2J=7.6 Hz, ^3J=2.3 Hz, 1H;H-5), 3.27(d, ^3J=2.0 Hz, 1H;OH), 3.24(dq,

^3J=6.9 Hz, ^3J=2.8 Hz, 1H;H-4), 2.11-1.95(m, 2H;H-9), 1.67-1.57(m, 1H;H-5'), 1.57-1.00(m, 6H; H-6; H-7,H-8, H-5'), 1.39(s, 3H;C2'$-CH_3$), 1.31(s, 3 H;C2'$-CH_3$), 1.16(s, 3H;H-1), 1.10(s, 3H, C2-(CH_3)), 1.03(d, ^3J=7.0 Hz, 3H;C4-(CH_3)), 0.93(d, ^3J=6.7 Hz, 3H;C6-(CH_3)).

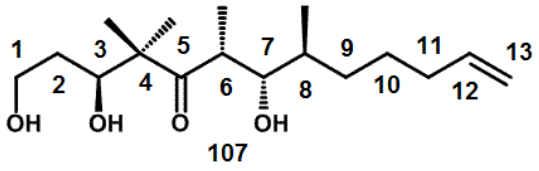
^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm):221.99 (s, C-3), 138.73 (d, C-10), 114.54 (t, C-11), 98.48 (s, C-2'), 74.71 (d, C-4'), 74.16 (d-C-5), 59.86 (t, C-6'), 51.52 (s, C-2), 41.5 (d, C-4), 35.38 (t, C-6), 33.86 (t, C-9), 32.20 (t, C-7), 29.69 (q, C2$-CH_3$), 26.08 (t, C-8), 25.27(t, C-5'), 21.43(t, C-1), 19.10 (q, C2-CH_3), 18.66 (q, C2'-CH_3), 15.44 (q, C6-CH_3), 10.64 (q, C4-CH_3),

IR(Film): $\widetilde{\nu}$(cm^{-1}) : 3504(w), 2928(s), 2857(s), 1685(s), 1459(s), 1381(s), 1372(s), 1329(w) 1308(w), 1272(m). 1255(m), 1197(s), 1161(m), 1107(s), 1056(w), 1019(m), 987(s), 971(s), 951(m) 854(m).

MS(EI) : m/z(%): 340(<1, [M^+]) , 325(5, [M^+-CH_3]), 307(3), 264(11), 243(4), 226(4), 199(5), 185(15), 156(55), 141(27), 137(12), 127(15), 115(53), 109(48), 99(25), 82(100), 69(52), 57(56).

HRMS(EI): calculated : 340.2614
found : 340.2615

5.5.2 (3*S*,6*R*,7*S*,8*S*)-1,3,7-Trihydroxy-4,4,6,8-tetramethyl-12-tridecen-5-one (107)



107

To a solution of acetonide protected diol **106a** (400 mg, 1.16 mmol) in CH_3CN (19.5 mL) was firstly added H_2O (2.77 mL) followed by addition of $CeCl_3.7H_2O$ (1.29 g, 3.45 mmol) at ambient temperature. The reaction was stirred at reflux temperature for 1.5 hr. The completion of reaction was monitored by TLC and after its completion, the reaction mixture was diluted with Et_2O, quenched with $NaHCO_3$ (saturated aq), and the aqueous layer was extracted with Et_2O (2X).

The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by using the flash chromatography (5:1, Pentane:Et_2O) and which provided 0.361 g of Triol **107** in 78% yield as clear oil.

General Data: $C_{17}H_{32}O_4$, FW:300.4, $[\alpha]_D^{20} = -45.5$(c=1.0, $CHCl_3$), TLC :R_f =0.17 (Pentane/Et_2O 3:1), UV($-$), Vanillin: black

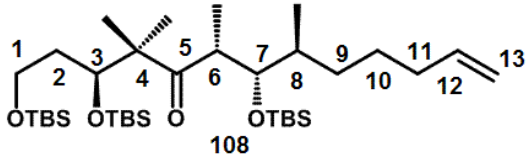
^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.81-5.65(m, 1H;H-10), 5.00-4.90(m, 2H;H-13), 4.04-4.01(m, 1H;H-3), 3.88-3.80(m, 2H;H-1), 3.35(br d, *J*=9.2 Hz,3H; H-7, C3-OH, C7-OH), 3.25(q, ^3J=6.9 Hz, 1H;H-6), 2.69 (br s,1H; C1-OH), 2.07-1.98(m,2H;H-11),1.78- 1.70(m,1H; H-9), 1.63-1.42(m, 4H; H-2, H-8, H-10), 1.35-0.96(m, 2H; H-9, H-10), 1.19,1.12(2S, 2 x 3H;C4-CH_3), 1.04(d , ^3J=6.9 Hz, 3H; C6-CH_3), 0.84(d, ^3J=6.8 Hz, 3H; C8-CH_3)

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm):221.99 (s, C-5), 138.73 (d, C-10), 114.54 (t, C-11), 76.4(d, C-3), 74.16 (d-C-7), 62.1 (t, C-1), 52.6 (s, C-4), 40.9 (d, C-6), 35.38 (t, C-8), 33.86 (t, C-2), 32.20 (t, C-9), 26.08 (t, C-10), 21.43(t, C4-CH_3), 18.66 (q, C4-CH_3), 15.44 (q, C8-CH_3), 10.64 (q, C6-CH_3)

IR(Film): $\widetilde{\nu}$(cm^{-1}) : 3419(br,s), 2935(s), 2882(s), 1685(s), 1470(s), 1383(m), 1329(m), 1056(s), 996(m) 910(m).

MS(EI) : m/z(%): 300(<1, [M^+])] 201 (13), 183 (19),165 (10), 156 (11), 127 (23), 109 (47), 100 (100), 82 (80), 69 (46), 57 (45), 43 (30);

5.5.3 (3*S*,6*R*,7*S*,8*S*)-1,3,7-Trihydroxy)-4,4,6,8-tetramethyl-12-tridecen-5-one (108)



108

2,6-Lutidine (1.61 mL, 13.8 mmol, 12 eq) and TBSOTf (1.57 mL, 6.9 mmol, 6 eq) were slowly added at - 78 °C to a solution of tri-alcohol **107** (345 mg, 1.15 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred at -78 °C for 30 minutes and at 0 °C for 1 hour. Saturated aqueous $NaHCO_3$ solution was added and the mixture was extracted with CH_2Cl_2. The combined extracts were dried over MgSO4 and concentrated in vacuo. Trisilyl ether **108** (730 mg, 90%) was obtained as a colorless oil after purification of the residue by flash chromatography (pentane/Et_2O 20:1).

General Data: $C_{35}H_{74}O_4Si_3$, FW:643.2, $[\alpha]_D^{20} = -41.0$(c=1.0, $CHCl_3$), TLC :R_f= 0.72(Pentane/Et_2O 10:1), UV$(-)$, Vanillin: black

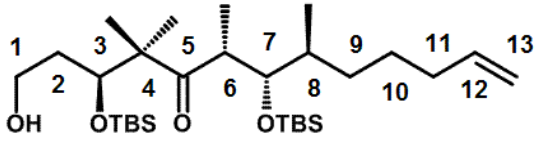
^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.83-5.73(m, 1H;H-12), 5.00-4.91(m, 2H;H-13), 3.88(dd, ^3J=7.6 Hz, ^3J=2.6 Hz, 1H;H-3), 3.76(dd, ^3J=6.7 Hz, ^3J=2.1 Hz, 1H;H-7), 3.69-3.63(m,1H;H-1), 3.59-3.53(m,1H;H-1), 3.15-3.11(m,1H;H-6), 2.05-1.99(m,2H;H-11) 1.58-1.37(m,5H) 1.19-1.11(m,2H)(H-2,H-8,H-9,H-10), 1.21(S,3H;C4-CH_3),1.03(d, ^3J=6.9 Hz, 3H; C6-CH_3) 1.01 (S,3H;C4-CH_3), 0.90(d, 3H;C8-CH_3), 0.89(2s, 2 x 9 H),0.87 (s,9H;OSiC$(CH_3)_3$), 0.08,0.05,0.02, 0.01(4S, 4 x 3 H;OSi$(CH_3)_2$),0.05(s,6H;OSi$(CH_3)_2$)

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm):221.99 (s, C-5), 138.73 (d, C-12), 114.54 (d, C-13), 77.71 (d, C-3), 74.0 (d-C-7), 61.0 (t, C-1), 53.52 (s, C-4), 45.0 (d, C-6), 38.9(t, C-8) 38.1 (t, C-2), 34.3 (t, C-9), 30.5 (t, C-10), 27.1 (q), 26.2(q), 26.1(q) 26.0 (q, OSi$(CH_3)_2$), 24.5(q, C4-CH_3), 19.10 (q, C4-CH_3), 17.66 (q, C8-CH_3), 15.44 (q, C6-CH_3), -3.7(q), -3.7(q) -3.8(q),-4.0(q),-5.2(q),-5.3(q,OSi$(CH_3)_2$)

IR(Film): $\tilde{\nu}$(cm^{-1}): 2957(s),2931(m), 2886(w), 2858(m), 1697(s), 1473(s), 1256(s), 1103(s), 987(s), 836(s), 775(s)

MS(EI) : m/z(%): 643(<1, [M^+])] 546 (2), 413 (3), 373 (8), 303 (100), 241 (54), 187 (9), 171 (16), 145 (28), 115 (19), 109 (98), 89 (84), 73 (64)

5.5.4 (3*S*,6*R*,7*S*,8*S*)-1,3,7-Tri-(*tert*-butyldimethylsilyloxy)-4-4,6,8-tetramethyl-1-2-tridecen-5-one (109)



109

To a solution of trislylether **108** (602 mg, 0.937 mmol) in THF (21 mL) was added to solution (1.68 mL HF.Py in 4.2 mL pyridine mixed 8.4 mL THF at 0 °C. The resulting reaction mixture was warmed to 25 °C by removing the ice-bath and allowed to stir at that temperature until starting material was gone. Saturated $NaHCO_3$ solution was added to quench the reaction and two layers were separated. The aqueous layer was extracted with ethyl acetate (84 mL x 3). The combined organic extracts were dried over $MgSO_4$ and the solvents were removed under reduced pressure. The crude product obtained was purified by using column chromatography over silica gel with pentane/diethylether (10:1) as eluent to yield the desired primary alcohol **109** (327 mg, 70%) as pale yellow oil.

General Data: $C_{20}H_{36}O_4$, FW:528.40, $[\alpha]_D^{20} = -23.6$(c=1.0, $CHCl_3$), TLC :R_f =0.17(Pentane/Et_2O 3:1), UV$(-)$, Vanillin: black

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.83-5.73(m, 1H;H-12), 5.00-4.91(m, 2H;H-13), 3.88(dd, ^3J=7.6 Hz, ^3J=2.6 Hz, 1H;H-3), 3.76(dd, ^3J=6.7 Hz, ^3J=2.1 Hz, 1H;H-7), 3.69-3.63(m,1H;H-1), 3.15-3.11(m,1H;H-6), 2.05-1.99(m,2H;H-11), 1.87-1.85 (m, 1H, OH), 1-61-1.55(m, 2H, H-2), 1.58-1.37(m,5H), 1.48-1.30 (m,3H), 1.19-1.11(m,2H)(H-2,H-8,H-9,H-10), 1.21(s,3H;C4-CH_3),1.03(d, ^3J=6.9 Hz, 3H; C6-CH_3) 1.01 (S,3H;C4-CH_3), 0.90(d, 3H;C8-CH_3), 0.89(2s, 2 x 9H),0.87 (s,9H;OSiC(CH_3)$_3$), 0.08,0.05,0.02, 0.01(4S, 4 x 3 H;OSi(CH3)2),0.05 (s,6H;OSi(CH_3)$_2$);

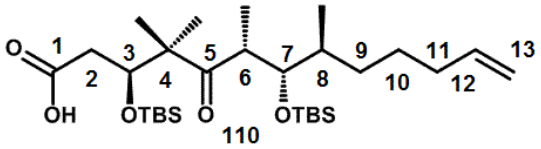
^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm):219.6 (s, C-5), 138.9 (d, C-12), 114.5 (d, C-13), 77.71 (d, C-3), 73.4 (d-C-7), 60.0 (t, C-1), 53.5 (s, C-4), 45.1 (d, C-6), 40.9(t, C-8) 38.7 (t, C-2), 34.3 (t, C-9), 30.3 (t, C-10), 27.0(q), 26.2(q), 26.2(q)

26.0 (q, $OSi(CH_3)_2$), 24.0(q, C4-CH_3), 18.4, 18.3 (q, C4-CH_3), 17.7, 17.7 (q, C8-CH_3), 15.7 (q, C6-CH_3), -3.6(q), -3.8(q) -3.9(q) (q,$OSi(CH_3)_2$)

IR(Film): $\widetilde{\nu}(cm^{-1})$ 3444(s), 2957(s), 2931(s), 2886(w), 2858(w), 1693(s), 1463(w), 1255(s), 1094(br,s), 987(w), 836(s), 775(s)

MS(EI) : m/z(%): 472(3)([M^+-tBu])] 413 (4), 345 (11), 299 (4), 271 (10), 241(48), 189 (100), 145 (26), 109 (90), 75 (46), 73 (63)

5.5.5 (3*S*,6*R*,7*S*,8*S*)-3,7-Di-(*tert*-butyldimethyl- silyloxy)-4,4-6,8-tetramethyl-5-oxo-12-tridecen-5-oicacid (110)



110

A solution of PDC (1.03 g, 2.73mmol, 11.0 eq) in DMF (3 mL) was added to a solution of alcohol **109** (120 mg, 0.226 mmol) in DMF (2 mL). The reaction mixture was stirred for 36 h at room temperature, mixed with brine (50 mL), diluted with water, and extracted with CH_2Cl_2. The combined extracts were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (pentane/Et_2O 2:1) to furnish acid **110** (84.9 mg, 68 %) as a viscous, colorless oil.

General Data: $C_{20}H_{36}O_4$, FW:542.9, $[\alpha]_D^{20} = -31.8$(c=1.0, $CHCl_3$), TLC :R_f =0.17(Pentane/Et_2O 3:1), UV($-$), Vanillin: black

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.83-5.73(m, 1H; H-12), 5.00-4.91(m, 2H; H-13), 3.88(dd, ^3J=7.6 Hz, ^3J=2.6 Hz, 1H; H-3), 3.76(dd, ^3J=6.7 Hz, ^3J=2.1 Hz, 1H; H-7), 3.69-3.63(m, 1H; H-1), 3.59-3.53(m, 1H; H-1), 3.15-3.11(m, 1H; H-6), 2.05-1.99(m, 2H; H-11), 1.58-1.37(m,5H), 1.19-1.11(m,2H)(H-2, H-8, H-9, H-10), 1.21(S, 3H; C4-CH_3), 1.03(d, ^3J=6.9 Hz, 3H; C6-CH_3), 1.01 (S, 3H; C4-CH3), 0.90(d, 3H;C8-CH_3), 0.89(2s, 2 x 9 H),0.87 (s,9H;$OSiC(CH_3)_3$), 0.08,0.05, 0.02, 0.01(4S, 4 x 3 H;$OSi(CH_3)_2$), 0.05(s,6H;$OSi(CH_3)_2$);

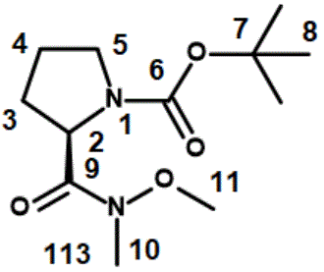
^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm):218.2 (s, C-5), 178.0 (s,C-1) 138.9 (d, C-12), 114.5 (d, C-13), 77.6 (d, C-3), 73.4 (d-C-7), 53.5 (s, C-4), 45.2(d, C-6), 40.2(t, C-2) 38.7 (t, C-8), 34.3 (t, C-9), 30.3 (t, C-10), 27.0(q), 26.2(q), 26.0 (q, $OSi(CH_3)_2$), 23.0(q, C4-CH_3), 19.1 (q, C4-CH_3), 18.2, 17.7 (q, C8-CH_3), 15.7 (q, C6-CH_3), -3.6(q), -3.8(q) -4.3(q),-4.6 (q,$OSi(CH_3)_2$

IR(Film): $\widetilde{\nu}$(cm^{-1}) max 2957(w), 2931(s), 2858(w), 1713(s), 1473(m), 1389(m), 1361(m), 1303(m), 1254(m), 1092(br, s), 989(w), 836(s), 776(s)

MS(EI) **:** m/z(%): 528 (<1) $[M+H-CH_3]^+$ 359 (14), 353 (13), 283 (14), 241 (20), 203 (100), 185 (12), 149(16), 115 (58), 109 (32), 75 (36), 73 (51)

5.6 Synthesis of Amino Acids Fused Benzimidazole Rings

5.6.1 *(S)-tert*-Butyl 2-(N-methoxy-N-methylcarbamoyl)-pyrrolidine-1-carboxylate (113)



Boc-L-proline (5.0 g, 20.38 mmol, 1.0 eq), *N,O*- dimethylhydroxylamine-HCl (2.64 g, 27.11 mmol, 1.33 eq) and DMAP(320 mg, 2.61 mmol, 0.129 eq) were added to an oven dry 250 mL round bottom flask. Dry CH_2Cl_2 (102 mL) was added and the reaction mixture was cooled to 0 °C via an ice bath. Then, triethylamine (4.75 mL, 33.92 mmol, 1.67 eq) was added slowly to the reaction mixture over 5 min followed by the addition of EDCI (4.68 g, 24.45 mmol, 1.2 eq). The solution was allowed to stir for 1 hour at 0 °C. The ice bath was then removed and the reaction mixture was stirred for 19 hours at room temperature. The reaction mixture was transferred to separatory funnel and washed with 1 N HCl (2 x 34 mL), sat. $NaHCO_3$ (2 x 34 mL), and saturated NaCl (2 x 34 mL). The organic layers were dried over Na_2SO_4, concentrated on a rotary evaporator, and dried under vacuum to give 80% yield of Weinreb product **113** (3.53g) as colorless oil.

General Data: $C_{12}H_{22}N_2O_4$, FW: 258.31, TLC :R_f=0.17(Pentane/EtOAc 1:1), UV(−), Vanillin: brown

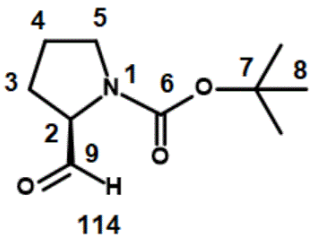
^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm):4.67 – 4.65 (m, 2H,H-5), 4.57 – 4.55 (m, 2H,H-5), 3.74 (s, 1.4H), 3.68 (s, 1.6H), 3.55 – 3.35 (m, 2H, H-3), 3.16 (s, 3H), 2.17 – 2.04 (m, 1H), 1.94 – 1.77 (m, 3H), 1.42 (s, 4H), 1.38 (s, 5H)

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm)173.9 (C, C-9), 173.3 (C, C-9), 154.5 (C, C-6), 153.9 (C, C-6), 79.5 (C-7), 79.4(C-7), 61.3 (C-2), 61.2 (C-2), 56.8 (CH3,C-11), 56.5 (CH3, C-11), 45.9 (CH2, C-5), 46.6 (CH2, C-5), 32.4 (CH3,

C-10), 32.3 (CH3,C-10), 30.5 (CH2,C-3), 29.6 (CH2,C-3), 28.5 (CH3, C-8), 28.4 (CH3, C-8), 24.1 (CH2,C-4), 23.4 (CH2, C-4)

IR(Film): $\widetilde{\nu}(\mathrm{cm}^{-1})$: 2971,2934, 2877, 1689, 1387, 1159, 1152.

5.6.2 *(S)-tert*-Butyl 2-formylpyrrolidine-1-carboxylate (114)



114

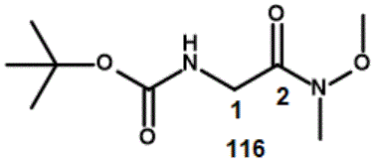
Boc-proline-Weinreb amide **113** (3.20 g, 12.3 mmol, 1.0 eq) was added to an oven dry round bottom flask, followed by dry THF (103 mL) addition. The reaction solution was cooled to 0 °C via an ice bath and followed by addition of solid $LiAlH_4$ (586 mg, 15.4 mmol, 1.25 eq), which was carefully carried out in three equal portions over 1 min. The solution was stirred for another 40 min at 0 °C and then 0.4 M solution of $NaHSO_4$ (2.99 g, 21.63 mmol 1.75 eq) was prepared and cooled to 0 °C. After 40 min of stirring, the reaction mixture was quenched with a slow addition of 0.4 M $NaHSO_4$ (57 mL). The ice bath was removed and the solution was stirred for another 12 min. The mixture was transferred to a separatory funnel, washing with H_2O (86 mL), and the aqueous layer was extracted with EtOAc (4 x 59 mL). The organic layers were combined and washed with 1 N HCl (3 x 52 mL), sat. $NaHCO_3$ (2 x 52 mL), and sat. NaCl (2 x 52 mL). After the series of aqueous washes, the organic layers were dried over Na_2SO_4, concentrated on a rotary evaporator carefully, and dried under vacuum (30 min) to give 1.83 g (75%) of **114** as a clear oil.

General Data: $C_{10}H_{17}NO_3$, FW:199.24, TLC :R_f=0.17(Pentane/EtOAc 1:1), UV(−), Vanillin: brown

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm)) 1.39 (9H, s), 1.75-1.94 (3H, m), 2.03-2.12 (1H, m), 3.33-3.44 (2H, m), 4.08 (1H, ddd, ^2J= 8.2, ^3J=5.5, ^3J=2.6 Hz), 9.45 (1H, d, J=2.6 Hz).

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm) 101 MHz, C6D6, 1:1 mixture of rotamers) 23.7 (CH2, C-4), 24.6 (CH2, C-4), 26.6 (CH2, C-3), 27.8 (CH2, C-3), 28.3 (CH3, C-8), 28.4 (CH3, C-8), 46.8 (CH2, C-5), 46.9 (CH2, C-5), 65.1 (t,), 65.2 (t, C-2), 79.6 (S, C-7), 79.8 (S, C-7), 153.7 (S, C-6), 154.7 (S, C-6), 199.5 (S, C-1), 199.8 (S, C-1).

5.6.3 *tert*-Butyl N-[2-[methoxy(methyl)amino]-2-oxo-ethyl]-carbamate (116)



116

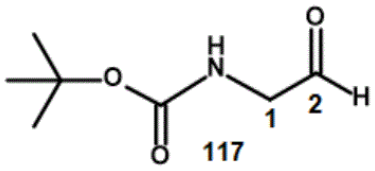
Boc-glycine (5.0 g, 28.55 mmol, 1.0 eq), *N,O* dimethylhydroxylamine-HCl (3.71 g, 38.07 mmol, 1.33 eq), and DMAP (352 mg, 2.85 mmol, 0.1 eq) were added to an oven dry round bottom flask. Dry CH_2Cl_2 (143 mL) was added and the solution cooled to 0 °C via an ice bath. Then, triethylamine (4.7 mL, 34.26 mmol, 1.2 eq) was added slowly to the reaction mixture over 5 min followed by the addition of EDCI (6.56 g, 34.26 mmol, 1.2 eq). The solution was allowed to stir for 1 hour at 0 °C. The ice bath was then removed and the reaction mixture was stirred for 19 hours at room temperature. The reaction mixture was transferred to a separatory funnel and washed with 1 N HCl (2 x 50 mL), sat. $NaHCO_3$ (2 x 50 mL), and saturated NaCl (2 x 50 mL). The organic layers were dried over Na_2SO_4, concentrated on a rotary evaporator and dried under vacuum to give 80% yield of Weinreb product **116** (4.79 g) as colourless oil.

General Data: $C_9H_{18}N_2O_4$, FW: 218.2, TLC **:**R_f=0.20(Pentane/EtOAc 1:1), UV(−), Vanillin**:** brown

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.34 (br s, Carbamate-NH), 4.08(s, 2H, H-1), 3.72 (s, 3H, OCH_3), 3.20 (s, 3H, NCH_3), 1.42(s, 9H, *t*-butyl-CH_3)

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm)173.9 (C), 154.5 (C), 79.5 (C), 56.5 (CH_3), 41.9 (CH_2), 32.4 (CH_3), 28.5 (CH_3)

5.6.4 *tert*-Butyl N-(2-oxoethyl)carbamate (117)



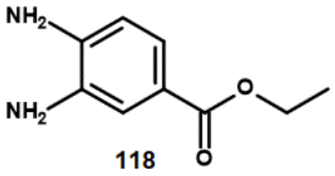
Boc-proline-Weinreb amide **116** (4.2 g, 16.5 mmol, 1.0 eq) was added to an oven dry round bottom flask, followed by dry THF (137 mL). The reaction solution was cooled to 0 °C via an ice bath and $LiAlH_4$ (783 mg, 20.62 mmol, 1.25 eq) was carefully added as a solid in three equal portions over 1 min. The solution was stirred for 40 min at 0 °C and then 0.4 M solution of $NaHSO_4$ (3.99 g, 28.87 mmol 1.75 eq) was prepared and cooled to 0 °C. After 40 min of stirring, the reaction mixture was quenched with slow addition of 0.4 M $NaHSO_4$ (75 mL). The ice bath was removed and the solution was stirred for another 12 min. The mixture was transferred to separatory funnel, washing with H_2O (114 mL), and the aqueous layer was extracted with EtOAc (4 x 73 mL). The organic layers were combined and washed with 1 N HCl (3 x 73 mL), sat. $NaHCO_3$ (2 x 73 mL), and sat. NaCl (2 x73 mL). After the series of aqueous washes, the organic layers were dried over Na_2SO_4, concentrated on a rotary evaporator carefully, and dried under vacuum (30 min) to give 2.70 g (75%) of aldehyde **117** as a clear oil.

General Data: $C_7H_{13}NO_3$, FW: 159.2, TLC :R_f=0.21(Pentane/EtOAc 1:1), UV(−), Vanillin: brown

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 5.34 (br s, Carbamate-NH), 4.08(s, 2H, H-1), 3.72 (s, 3H, OCH_3), 3.20 (s, 3H, NCH_3), 1.42(s, 9H, *t*-butyl-CH_3)

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm)173.9 (C), 154.5 (C), 79.5 (C), 56.5 (CH_3), 41.9 (CH_2), 32.4 (CH_3), 28.5 (CH_3)

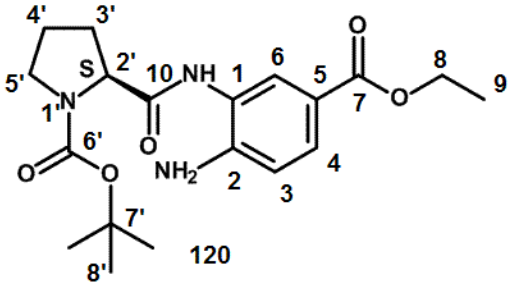
5.6.5 Ethyl 3,4-diaminobenzoate (118)



10 mL of $SOCl_2$ was added to anhydrous ethanol (100 mL) dropwise at below 0 °C. Then 3,4-diaminobenzoic acid (1.5g, 1.0 mmol) was added portion-wise and then reaction mixture was refluxed for 4 hours. Then methanol was evaporated under reduced pressure and the residue was washed with saturated $NaHCO_3$, filtered and dried. The whole process furnished 1.74 g (95% yield) of ethyl 3,4-diaminobenzoate **118**.

General Data: $C_9H_{12}N_2O_2$, FW: 180.24 , TLC :R_f=0.17(Pentane/EtOAc 1:1), UV(−), Vanillin: brown

5.6.6 *tert*-Butyl 2(*S*)-2-[(2-amino-5-ethoxycarbonyl-phenyl) carbamoyl]pyrrolidine-1-carboxylate (120)



N-Methylmorpholine (0.244 mL, 2.22 mmol) was added dropwise over 5 min to a stirred solution of Boc-L-proline (0.573g, 2.21 mmol, 1eq) in CH_2Cl_2 (20 ml) at 0 °C under an atmosphere of nitrogen. The solution was stirred at -10 °C for 5 min, then isobutyl chloroformate (0.287 mL, 2.21 mmol. 1eq) was added dropwise over 5 min and the mixture was stirred at -10 °C for a further 10 min and then ethyl 3,4 diaminobenzoate **118** (0.905 g, 2.65 mmol, 1.2eq) was added portion-wise over 5 min and the mixture was stirred at -10 °C for 2 h and then at room temperature for 44 h. The solvent was evaporated by using rotary then crude product was purified by using flash chromatography

and petane/ethylacetate $1{:}1$ as eluent resulting into amide **120** (0.585 g) having 81% yield.

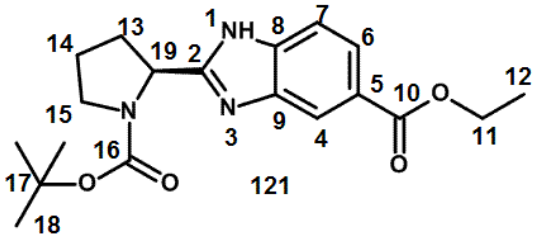
General Data: $C_{19}H_{27}N_3O_5$, FW:377.19, $[\alpha]_D^{20} = +32.5$(c=1.0, $CHCl_3$), TLC :R_f=0.17(Pentane/Ethylacetate 1:1), UV$(+)$, Vanillin: yellowish-brown

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 7.93(d,J=8.48 Hz, 1H; H-6), 7.25(S, lH; H-4), 4.38(q, 2H; H-8), 3.56-3.7 (m, 2H; H-5'), 2.48-2.08(m, 2H; H-3'), 2.0-2.08(m, 2H; H-4'), 1.49 (S, 9H; H-8'), 1.37(t, 3H; H-9).

^{13}C-NMR(100 MHz, CH_3OD): δ(ppm) 171.17(S, C-10), 167.13(S, C-7), 156.64 (S, C-6'), 124.13(d, C-4), 123.93(S, C-6), 118.5(d, C-5), 115.30 (d, C-3), 80.7(S, C-7'), 60.66(d, C-2'-CH), 54.60(q, C-8-CH_2) 47.29(t, C-5'-CH_2), 34.13(t, C-3'-CH_2), 28.16(S, C-8'-CH_3), 22.30(d, C-4'-CH_2), 14.19 (t, C-9-CH_3)

MS(EI) : m/z(%): 377.1(<1, [M^+]), 324(3) 332(16), 321(21), 304.1(11), 277.1(6, [M^+]-BOC), 258.1(14), 232.1(3), 217.1(8), 207.1(92), 180.1(100), 161.0(16), 151.0(26), 135.1(15), 114.0(77), 107.1(8), 79.0(4), 70.1(98), 57.0(68).

5.6.7 Ethyl 2-((*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)-3H-benzo[d]imidazole-5-carboxylate (121)



The amide **120** (0.647 g, 1.71mmol) was dissolved in 4 mL of DMSO and 12 ml of acetic acid and were stirred for 12 hours at 75 °C. Then the reaction mixture was allowed to cool down and neutralized by using 1 molar solution of NaOH then was partitioned between ethylacetate and water. The organic layer was washed with aqueous $NaHCO_3$ ((3 x 30 mL)and water (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (eluting solvent pentane/ethyl acetate1:1) to provide the desired product **121** (0.585 g) having 95% yield.

General Data: $C_{19}H_{25}N_3O_4$, FW:359.4, $[\alpha]_D^{20} = +32.5$(c=1.0, $CHCl_3$), TLC :R_f=0.17(Pentane/EtOAc 1:1), UV(+), Vanillin: yellow

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 8.22(s, 1H), 7.93(d,J=8.7 Hz, 1H), 7.57(d, IH), 4.38(q, 2H), 3.56-3.7 (m, 2H), 2.48-2.08(m, 2H), 2.0-2.08(m, 2H), 1.41 (t, 3H), 1.13(s, 9H)

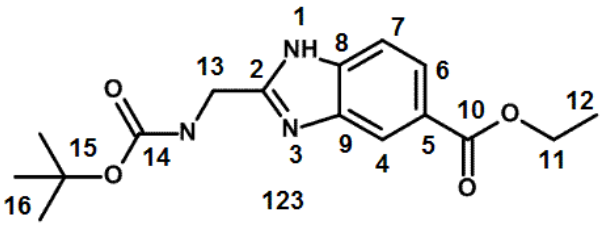
^{13}C-NMR(100 MHz, $CDCl_3$): Rotamers δ(ppm) 172.93, 172.54 (S, C-16), 168.5, 167.22(S, C-10), 161.42, 160.81(S, C-2), 156.55, 155.85(S, C-9), 142.75, 139.4(S, C-5), 125,9, 125.8(d, C-8), 125.04, 124.92(d, C-6), 115.30 (d, C-7), 81.55, 81.41(S, C-17), 62.1 (q, C-11), 57.70, 57.19 (d, C-19), 48,387, 47.964(d,C-15), 34.8, 33.61(d, C13), 25.363, 24.807(d, C-14), 14.7(t, C-12)

IR(Film): $\widetilde{\nu}$(cm^{-1}): 3234.34, 2979.34, 2371.60, 2346.74, 1711.81, 1625.44, 1585.30, 1535.20, 1477.97, 1453.72, 1395.49, 1367.85, 1306.53, 1238.68, 1207.09, 1161.62, 1123.40, 1088.05, 1020.14, 971.05, 948.23, 918.54, 900.20, 877.00, 830.62, 772.30, 755.53, 668.61, 585.42, 559.67, 537.27, 517.58, 461.96, 417.87

LC-MS: m/z(%): 359.0(0.8) [M-H^-], 358.2(3.9), 227(1.8), 113.0(7.5)

5.6.8 Ethyl 2-((*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)-3H-benzo[d]imidazole-5-carboxylate (123)

.



N-Methylmorpholine (1.0 mL, 9.09 mmol. 1eq) was added dropwise over 5 min to a stirred solution of Boc-glycine (1.7g, 9.09mmol, 1eq) in CH_2Cl_2 (20 mL) at -10 °C under an atmosphere of nitrogen. The solution was stirred at -10 °C for 5 min, then isobutyl chloroformate (1.17 mL, 9.09 mmol, 1eq) was added dropwise over 5 min and the mixture was stirred at -10 °C for a further

10 min and then then ethyl 3,4 diaminobenzoate **118** (3.77 g, 9.99 mmol, 1.1eq) was added portion-wise over 5 min and the mixture was stirred at -10 °C for 2 h and then at room temperature for 44 h. The solvent was evaporated by using rotary then crude product was purified by using flash chromatography and pentane/ethylacetate 1:1 as eluent resulting into amide **122** (2.45 g) having 80% yield.

The amide **122** (0.576g, 1.71mmol) was dissolved in 3 mL of DMSO and 9 mL of acetic acid and was stirred for 12 hours at 75 °C. Then the reaction mixture was allowed to cool and subsequently neutralized by using 1 molar solution of NaOH then was partitioned between ethylacetate and water. The organic layer was washed with aqueous $NaHCO_3$ ((3 x 30 mL)and water (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (eluting solvents pentane/ethyl acetate 1:1) to provide the desired product **123** (0.513g) having 94% yield.

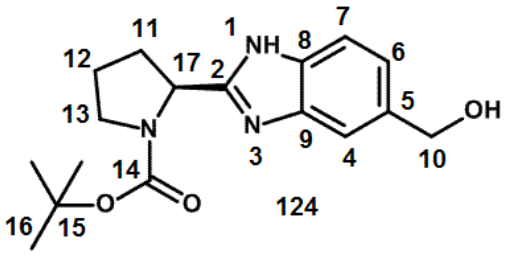
General Data: $C_{16}H_{21}N_3O_4$, FW:319.36, TLC **:**R_f=0.17(Pentane/EtOAc 1:1), UV$(+)$, Vanillin**:** yellow

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 8.26(s, 1H), 7.52(d,J=8.7 Hz, 1H), 7.33(d, IH), 4.57(d,1-H) 4.36(q, 2H), 1.37(s, 9H), 1.22(t, 3-H)

^{13}C-NMR(100 MHz, $CDCl_3$): δ(ppm) 167.03(S, C-10), 157.2(S, C-14), 154.7 (S, C-2), 141.75 (m, C-9), 139.0(S, C-8), 127,4(m, C-4), 124.1(m, C-6), 114.5 (d, C-7), 80.4(S, C-15), 60.7 (q, C-11), 38.9(d, C13), 28.2(d, C-16), 14.4(t, C-12)

LC-MS: m/z(%): 319.2(20) [M+H$^+$], 219.0(10), 242(12)

5.6.9 2-(6-Hydroxymethyl-1H-benzoimidazol-2-yl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (124)



124

A 100-mL, two-necked, round-bottomed flask is equipped with a magnetic stirring bar, reflux condenser bearing a drying tube and a pressure-equalizing dropping funnel fitted with a rubber septum. The flask is charged with 17 mL of tetrahydrofuran (THF) and (0.180 g, 4.75 mmol, 1.9eq) of lithium aluminum hydride(LAH). The solution of benzimidazole ester **121** (0.570g, 1.58 mmol. 1eq) in THF (2 mL) is added dropwise to the stirred suspension in the flask. The dropping syringe is washed 1-mL of THF two times and the suspension is stirred for an additional 20 min, when TLC analysis shows the complete formation of the benzimidizole alcohol **124**.

The 1.66 mL of a 10% aqueous potassium hydroxide solution is added drop wise to the reaction mixture cooled with an ice-water bath. The mixture is stirred for 1 hr at room temperature, then the white precipitate is removed by filtration through a Celite pad and the pad is rinsed with three 20-mL portions of CH_2Cl_2. The combined organic filtrates are washed with 2.5 mL of aqueous phosphate buffer (pH 7), and the aqueous layer is extracted with CH_2Cl_2 (3 x 10 mL). The combined organic phases are dried with anhydrous sodium sulfate and concentrated under reduced pressure to give 0.451 g alcohol **124** (90% yield) of pale yellow oil.

General Data: $C_{17}H_{23}N_3O_3$, FW:317.38, $[\alpha]_D^{20} = +32.5$(c=1.0, $CHCl_3$), TLC :R_f=0.027(Pentane/EtOAc 1:1), UV(+), Vanillin: yellow.

^{1}H-NMR(400 MHz, CH_3OD): δ(ppm): 7.38-7.50(m, 2H), 7.17(d, J=0.021 Hz, 1H), 5.77(m, 1H), 5.07(m, 2H), 4.78(dt, 1H), 3.43-3.50(m, 2H), 2.85-2.90(m, 1H),2.54-2.35(m,2H) 1.98-2.10(m, 2H), 1.28(s, 9H).

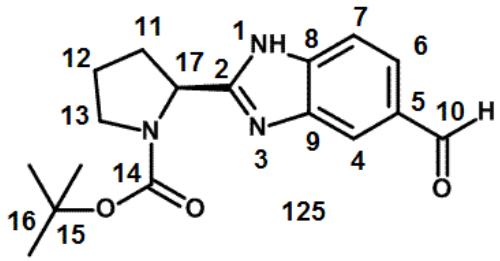
^{13}C-NMR(100 MHz, CH_3OD): δ(ppm) 158.84 (S, C-14), 156.46(S, C-2), 139.26 (S, C-9), 137.28(S, C-5), 123.14(d, C-6), 115.60(d, C-4), 114.30 (d, C-7),

81.37(S, C-17), 65.65(t, C-15), 57.65(d, C-2), 47.964(t,C-15), 34.82(t, C-11), 30.18(S, C-16), 28.7(S, C-16)

IR(Film): $\widetilde{\nu}(\mathrm{cm}^{-1})$: 3150.56, 2974.53, 2929.03, 2871.83, 2110.98, 1879.81, 1769.99, 1669.95, 1542.10, 1476.05, 1450.04, 1404.20, 1366.26, 1324.94, 1301.08, 1277.79, 1248.5, 1202.80, 1158.64, 1125.51, 1090.31, 1039.37, 1004.49, 942.76, 798.64, 775.93, 730.29, 666.49.

MS$(\boldsymbol{EI})$ **:** m/z(%): 318.174(<1, $[M^+]$), 315.37(74) 279.38(1), 259(75), 242.30 (28), 231.32(1), 214.29(23, $[M^+]$-BOC), 197.26(10), 187.25(15), 173.24(100), 160.22(38), 147.20(7), 131.20(4), 111.23(3)

5.6.10 2-(6-Formyl-1H-benzoimidazol-2-yl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (125)



125

A mixture of proline fused benzimidazole alcohol **124** (.400g, 1.26 mmol, 1eq) and MnO_2 (0.547 g, 6.30 mmol, 5eq) in CH_2Cl_2 (30 mL) was stirred at room temperature for 20 hours. The completion of reaction was monitored with TLC and then reaction mixture was filtered over Celite.The Celite was rinsed with CH_2Cl_2 (3 x 20 mL). The filtrate solvent was evaporated under reduced pressure and pure product **125** (0.378 g) by having 95% yield as light yellow coloured viscous oil was obtained.

General Data: $C_{17}H_{21}N_3O_3$, FW:315.15, $[\alpha]_D^{20} = +31.3$(c=1.0, $CHCl_3$), TLC :R_f=0.20(EtOAc/Pentan 1:3), UV$(+)$, Vanillin: yellow

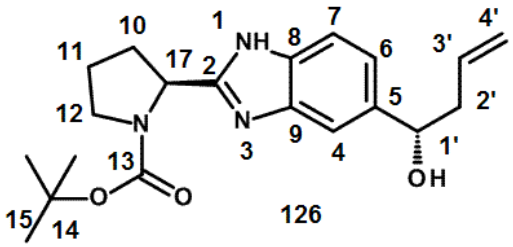
^{1}H-NMR(400 MHz, CD_3OD): δ(ppm): 8.10(S, 1H),7.80(d,J=6.32 Hz, 1H), 5.16(d,J=6.12 Hz, 1H), 3.53 (m, 2H), 2.22-2.31(m, 2H), 2.0-2.10(m, 2H), 1.28(s, 9H)

^{13}C-NMR(100 MHz, CD_3OD): δ(ppm) 191.89 (S, C-10), 156.46(S, C-2), 141.87 (S, C-9), 138.28(S, C-8), 131.82(S, C-5), 124.60(d, C-6), 115.30 (d, C-7), 81.37(S, C-15), 57.74(d, C-2), 47.4(t,C-13), 34.82(t, C-12), 28.80(S, C-16), 22.26(t, C-11).

IR(Film): $\tilde{\nu}$(cm^{-1}): 3430.33, 3187.95, 2975.54, 2933.23, 2881.87, 1677.97, 1619.51, 1589.57, 1534.62, 1477.97, 1449.40, 1392.58, 1366.41, 1324.90, 1303.08, 1284.54, 1255.7, 1159.19, 1121.70, 1089.54, 1036.01, 1004.79, 971.23, 961.51, 918.44, 899.74, 876.83, 851.48, 803.90, 772.46, 687.13.

MS(EI) : m/z(%): 316.156(<1, [M$^+$]), 315.37(74) 279.38(1), 259(75), 242.30 (28), 231.32(1), 214.29(23, [M$^+$]-BOC), 197.26(10), 187.25(15), 173.24(100), 160.22(38), 147.20(7), 131.20(4), 111.23(3).

5.6.11 *tert*-Butyl(2*S*)-2-[5-[(1*S*)-1-hydroxybut-3-enyl]-1H-benzimidazol-2-yl]pyrrolidine-1-carboxylate (126)



126

Allyl magnesium bromide (1.6 mL, 1.61 mmol, 1.7 eq) was added to a solution of (-)-DIP-Cl (0.516 g, 1.61 mmol, 1.7eq) in 5 mL of dry Et_2O . After stirring at room temperature for 1h, 4 mL of hexane was added to the solution and the formed precipitate was removed by filtration under argon. After washing with hexane (2 x 1 mL), the filtrate was cooled to -78 °C. In a second flask aldehyde **125** (0.300g, 0.948 mmol, 1eq) was suspended in 4 ml of freshly distilled dry Et_2O and cooled to -100 °C. The cooled borane solution was slowly added to this suspension via syringe and the solution was stirred at -100 °C for another 90 min after the completion of addition. Then 0.302 mL of dry MeOH were added and the mixture was warmed to 10 °C.

The reaction mixture was left stirring overnight after the addition of 0.59 mL of ethanolamine. Then, the reaction mixture pH was adjusted to 8 with water and saturated aqueous NH_4Cl and subsequently the aqueous phase was extracted three times with 20 mL of CH_2Cl_2. The combined organic extracts were then dried over $MgSO_4$ and the solvent was removed under reduced pressure. The flash chromatography with ethylacetate/pentane 1:1 furnished the desired homoallylic alcohol **126** as a viscous oil (0.308 g, 91%, ee 91% determined by MTPA ester analysis).

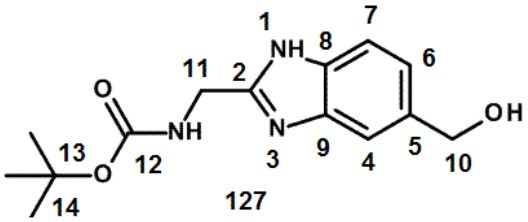
General Data: $C_{20}H_{27}N_3O_3$, FW:357.45, $[\alpha]_D^{20} = -35.3$(c=1.0, $CHCl_3$), TLC :R_f=0.17(EtOAc), UV$(+)$, Vanillin: yellow

^{1}H-NMR(400 MHz, CD_3OD): δ(ppm): 7.38-7.50(m, 2H), 7.17(d, J=0.021 Hz, 1H), 5.77(m, 1H), 5.07(m, 2H), 4.78(dt, 1H), 3.43-3.50(m, 2H), 2.85-2.90(m, 1H), 2.54-2.35(m, 2H), 1.98-2.10(m, 2H), 1.28(s, 9H).

^{13}C-NMR(100 MHz, CD_3OD): δ(ppm) 156.46(S, C-2), 139.01(S, C-9), 138.28 (S, C-8), 134.66(d, C4‘), 120.75(S, C-5), 117.95(t, C3‘), 114.60(d, C-6), 112.30(d, C-7), 80.80(S, C-15), 73.56(t, C1‘) 54.64(d, C-2), 47.36(t,C-13), 44.09(t, C-12), 28.74(S, C-16), 24.7(t, C-11).

MS(EI) **:** m/z(%): 358.20(<1, [M^+]), 357.42(74) 279.38(1), 259(75), 242.30 (28), 231.32(1), 214.29(23, [M^+]-BOC), 197.26(10), 187.25(15), 173.24(100), 160.22(38), 147.20(7), 131.20(4), 111.23(3)

5.6.12 *tert*-Butyl N-[[5-(hydroxymethyl)-1H-benzimidazol-2-yl]methyl]carbamate (127)



127

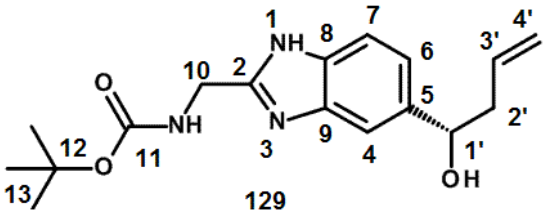
A two-necked round-bottomed flask equipped with a magnetic stirring bar and reflux condenser bearing a drying tube is charged with 20 mL of tetrahydrofuran (THF) and 0.180 g (4.75 mmol, 3eq) of lithium aluminum hydride (LAH). While the suspension in the flask is stirred, a solution of the benzimidazole ester **123** (0.504g, 1.58 mmol. 1eq) in THF (2 mL) is added dropwise. The dropping funnel is washed with two 3-mL portions of THF and the suspension is stirred for an additional 20 min, when TLC analysis shows the complete formation of the benzimidizole alcohol **127**.

The reaction mixture is cooled with an ice-water bath while 1.66 mL of a 10% aqueous potassium hydroxide solution is added dropwise. The mixture is stirred for 1 hr at room temperature, then the white precipitate is removed by filtration through a Celite pad and the pad is rinsed with three 20 mL portions of CH_2Cl_2. The combined organic filtrates are washed with 2.5 mL of aqueous phosphate buffer (pH 7), and the aqueous layer is extracted with CH_2Cl_2 (3 x 10 mL). The combined organic phases are dried with anhydrous sodium sulfate and concentrated under reduced pressure to give 0.398 g alcohol **127** (91% yield) of pale yellow oil.

General Data: $C_{14}H_{19}N_3O_3$, FW:277.3, TLC :R_f=0.20(EtOAc/Pentane 1:1) TLC :R_f=0.20(EtOAc/Pentane 1:3), UV$(+)$, Vanillin: yellow

^{1}H-NMR(400 MHz, $CDCl_3$): δ(ppm): 7.29-7.36(m, 2H), 7.05(d, J=2.0Hz, 1H), 4.64(S, 2H), 4.41(d, 2H), 1.28(s, 9H).

5.6.13 *tert*-Butyl-N-[[5-[(1*S*)-1-hydroxybut-3-enyl]-1H-benzimidazol-2-yl]methyl]carbamate (129)



129

A mixture of **127** (0.348g, 1.26 mmol) and MnO_2 (0.550 g) in CH_2Cl_2 (40 mL) was stirred at room temperature for 12 h and then filtered over Celite. Then the Celite was rinsed with CH_2Cl_2 (3 x 20 mL). The filtrate was concentrated under reduced pressure and used further for the next reaction without characterization of the product **128**.

Allyl magnesium bromide (1.6 M, 1.61 mmol, 1.7eq) is added to (-)-DIP-Cl (0.516 g, 1.61 mmol, 1.7eq) in 5 mL of dry Et_2O. After stirring at room temperature for 1hour, 4 mL of dry hexane was added to the solution and formed precipitate was removed by filtration under argon. After washing with hexane (2 x 3 mL), the filtrate was cooled to -78 °C. In a second flask aldehyde **128** (0.261g, 0.948 mmol, 1eq) was suspended in 4 mL of freshly distilled dry Et_2O and cooled to -100 °C. The cooled borane solution was slowly added to this suspension via cannula and the solution was stirred at -100 °C for another 90 min after the completion of addition.

Then 0.302 mL of dry MeOH were added and the mixture was warmed to 10 °C. After the addition of 0.59 mL of ethanolamine the mixture was left stirring overnight. The pH was adjusted to 8 with water and saturated aqueous NH_4Cl and aqueous phase was extracted three times with 20 mL of CH_2Cl_2. The combined organic extracts were then dried over $MgSO_4$ and the solvent was removed under reduced pressure. Flash chromatography with ethylacetate/pentane$1{:}1$ furnished the desired homoallylic alcohol **129** as a viscous oil (0.276 g, 92%, ee 91% determined by MTPA ester analysis).

General Data: $C_{17}H_{23}N_3O_3$, FW:317.17 $[\alpha]_D^{20} = -29.5$(c=1.0, $CHCl_3$), TLC : R_f=0.15(EtOAc), UV$(+)$, Vanillin: yellow.

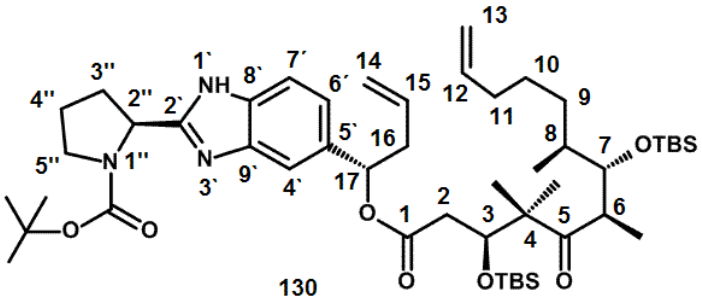
^{1}H-NMR(400 MHz, CH_3OD): δ(ppm): 7.41(s, 1H), 7.38(d, J=2.0 Hz, 1H), 7.14(d, J=2.0 Hz, 1H) 5.74(m, 1H), 5.03(t, 2H), 4.76(t, 1H), 4.42(d, 2H), 2.51(m, 2H), 1.37(s, 9H).

^{13}C-NMR(100 MHz, CH_3OD): δ(ppm) 156.46(S, C-11), 152.62(S, C-2), 138.92 (S, C-9), 137.67 (S, C-8) 134.76(d, C-4'), 124.75(S, C-5), 120.77 (d,C-6) 117.95(t, C3'), 114.89(d, C-7), 112.30(d, C-4), 80.80(S, C-12), 73.74(t, C1') 55.01(d, C-2), 43.96(t,C-10), 38.62(t, C-1'), 28.74(S, C-13).

LC-MS: m/z(%): [M+H^+] 318.2(95), [M-H^-] 316.2 (20)

5.7 Synthesis of Epothilone RCM-Substrate

5.7.1 *tert*-Butyl (2*S*)-2-[5-[(1*S*)-1-[(3*S*,6*R*,7*S*)-3,7-bis-[[*tert*-butyl(dimethyl)silyl]oxy]-4,4,6-trimethyl-5 -oxo-tri- dec-12-enoyl]oxybut-3-enyl]-1H-benzimid azol-2-yl]- pyrrolidine-1-carboxylate (130)



130

To the solution of alcohol **126** (26 mg, 0.072 mmol), carboxylic acid **110** (39 mg, 0.0718 mmol) and DMAP (3.91 mg, 0.0302 mmol) in 1 mL CH_2Cl_2 at 0 °C was added a solution of EDCI (18 mg, 0.093 mmol, 1.01eq) in 1 mL CH_2Cl_2 dropwise for 15 minutes and stirred for 24 hours at room temperature. The reaction mixture was poured into brine solution. The organic layer was separated, aqueous phase extracted three times, dried over $MgSO_4$ and concentrated under vacuum. The resulting residue was separated by silica gel column chromatography (5% solution of THF/CH_2Cl_2) to provide 44 mg (73%) of ester **130** as pale yellow oil.

General Data: $C_{49}H_{83}N_3O_7Si_2$, FW:882.37, $[\alpha]_D^{20}=-18.5$(c=0.02, $CHCl_3$), TLC :R_f=0.33(EtOAc/Pentane 1:3), UV$(+)$, Vanillin: Dark blue

^{1}H-NMR(400 MHz, CH_3OD): δ(ppm): 7.73-7.89(m, 2H, H-7',H$-4'$), 7.69(d, J = 0.11 Hz, 1H, H-6'), 5.92(m, 1H, H-2'), 5.77(m, 1H, H-15), 5.29(m,1H, H-13), 5.03-4.92(m, 4H, H-14, H-12), 4.34(dd, ^3J=4.9 Hz, ^3J=5.7 Hz, IH; H-3), 3.62-3.77(m, 2H, H-12'), 3.14(dq, ^3J=6.9 Hz, ^3J=6.7 Hz, IH; H-6), 2.85-2.90(m, 1H), 2.54-2.35(m, 2H), 1.98-2.10(m, 2H), 1.28(s, 9H), 1.04(d, ^3J=6.6 Hz, 3H; C6-CH_3), 0.90(dd, ^3J=6.6 Hz, 3H; C6-CH_3), 0.88 (2S, 2 x 9 H; OSiC$(CH_3)_3$), 0.061, 0.054, 0.049, 0.044, 0.029(4S, 4 x 3 H; OSi$(CH_3)_2$).

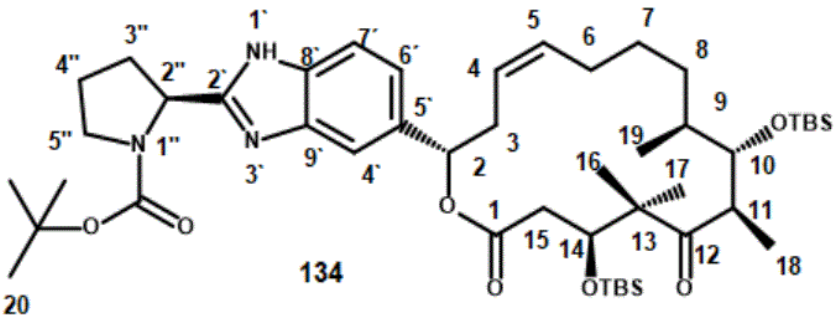
^{13}C-NMR(100 MHz, CH_3OD): δ(ppm) 217.6(S, C-5), 174.02(S, C-1),171.21 (S, C-13), 156.51(S, C-2̀), 138.91(S, C-12), 132.9(d, C-14), 128.98(d, C-8̀), 118.26(t, C-15), 114.37(t, C-13), 81.80(S, C-14̀),77.71(t, C-17), 75.51(d,C-7), 53.42(S, C-4), 47.44(t,C-12̀), 45.22(d, C-6), 40.9(t, C-16), 40.3(t,C-2), 38.74(d, C-8), 34.3(t, C-11), 31.8(t, C-10̀), 30.3(t, C-9), 28.41(S, C-15́), 27.1(t C-10), 26.1(q, C7-OSiC$(CH_3)_3$), 24.88(t, C-11̀) 23.2(q), 20.04(q, C4-CH_3), 18.49(s, C7-OSiC$(CH_3)_3$), 18.04(s, C3-OSiC$(CH_3)_3$), 17.68(q, C8-CH_3), 15.47(q, C6-CH_3), -3.64(q), -3.77(q, C7-OSi$(CH_3)_2$), -4.9(q), -5.0(q, C3-OSi$(CH_3)_3$)

IR(Film): $\widetilde{\nu}(cm^{-1})$: 3431.62 (br,m), 3077.69 (m), 2930.83 (s), 2930.83 (s), 2858.03 (s), 2738.59 (w), 2709.65 (w), 2247.49 (m), 1920.05 (w), 1827.29 (w), 1727.97 (s), 1689.2 (s), 1641.90 (m), 1619.65 (m), 1547.57 (w), 1472.96 (s), 1390.58 (s), 13 67.00 (s), 1304.14 (s), 1256.55 (s), 1173.89 (s), 1120.63 (s), 1088.45 (s),988.44 (s), 874.57 (s), 743.10 (s), 616.84(w), 567.03(w).

MS(EI) : m/z(%): 881.3(<1, [M$^-$]), 824.2(100), 768.1(52), 724.1(6), 655.1(3), 597.1(4), 556.0(4), 542.0(44), 486.0(4), 442.0(5), 427.1(11),399.1(24), 358.0(94), 316.0(20), 256.0(26), 185.0(28), 173.0(3), 109.0(7), 73.0(13), 57.0(16).

5.8 Epothilone Ring Closing Metathesis and Deprotections

5.8.1 *tert*-Butyl(2*S*)-2-[5-[(2*S*,4*Z*,9*S*,10*S*,11*R*,14*S*)-10,14-bis-[[*tert*-butyl-(dimethyl)silyl]ox]-9,11,13,13-tetramethyl-12,16-dioxo-1-oxacyclohexadec-4-en-2-yl]-1H-benzimidazol-2-yl]-pyrrolidine-1-carboxylate (134)



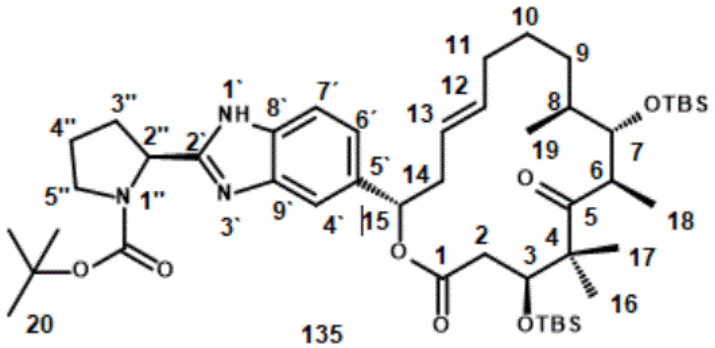
The diene **130** (24 mg, 0.027 mmol) was dissolved firstly in toluene (70 mL), heated to reflux and subsequently treated with a solution of 2nd generation Grubbs catalyst (3 mg, 3.6 μmol) in toluene (1 mL). The solution was stirred for 15 min and cooled to room temperature, filtered through a plug of silica and the precipitate was rinsed with toluene/MeOH 10/1. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 50/1) to give the desired macrolactones as inseparable mixture of *Z* (**134**) and *E* (**135**) isomers in 1:1 ratio as a yellow oil 19.7 mg and having 85% yield.

General Data: $C_{47}H_{79}N_3O_7Si_2$, FW:854.32, $[\alpha]_D^{20} = -13.5$(c=0.02, $CHCl_3$), TLC :R_f=0.32(Pentane/EtOAc 1:3), UV(+), Vanillin: Light-blue

^{1}H-NMR(400 MHz, CD_3OD): δ(ppm): Z-isomer 8.37(s,1H, H-4'), 7.68(d, J=3.2 Hz, 1H, H-7'), 7.25(m, 1H, H-6'), 5.72(m, 1H, H-5), 5.43(m, 1H, H-4), 5.32(m,1H, H-2"), 4.92(dd, ^3J=1.92 Hz, ^3J=7.96 Hz, IH, H-2), 4.32(dd, ^3J=2.0 Hz, ^3J=8.0Hz, IH, H-14), 3.50 (m,2H, H-5") 3.14(dq, ^3J=1.2 Hz, ^3J=6.4Hz, 2H, H-15), 3.09(dq, ^3J=8.1 Hz, ^3J=7.0 Hz, IH, H-11), 2.65(m,1H, H-3), 2.55(m, 2H, H-3") 2.49-2.35(m, 1H, H-7),2.05 (m, 2H, H-4") 1.87-1.82(m, 1H, H-7), 1.61-1.48(m, 3H, H-7, H-8, H-9), 1.28(s, 9 H, H-20) 1.19-1.00(m, 2H, H-6, H-8), 0.94(d, ^3J=8.0 Hz, 3H, H-19), 0.91, 0.89(2S, 2 x 9H, $OSiC(CH_3)_3$),0.08, 0.06, 0.03,-0.33(4s, 4 x 3 H, $OSi(CH_3)_3$).

^{13}C-NMR(100 MHz, CD_3OD): δ(ppm) Z-Isomer 218.6(S, C-12), 171.1(S, C-1), 159.2(d, C-2'), 142.1(d, C-5'), 135.1(d, C-5), 124.4(d, C-4) ,122.9(t, C-6'), 119.8(d, C-7'), 110.5(s, C-4'), 76.2(d, C-14), 73.3(d, C-2), 57.2(t, C-2"), 46.8(t, C-5") 44.5(d, C-11), 44.0(t, C-15),) 39.01(t,C-3), 32.5(t, C-3"), 30.39(t, C-6), 32.5(t, C-3"), 30.39(t, C-6), 29.2(t, C-8), 28.8(t, C-7), 27.72(S, C-20) 26.75, 26.13(q, -$OSiC(CH_3)_3$), 24.93(q, C-22), 24.8(q, C-17), 22.6(t, C-4"), 18.7(S, $OSiC(CH_3)_3$), 14. -4.12, -4.28, -4.89(q, $OSi(CH_3)_2$).

5.8.2 *tert*-Butyl(2*S*)-2-[5-[(2*S*,4*E*,9*S*,10*S*,11*R*,14*S*)-10,14-bis-[[*tert*-butyl-(dimethyl)silyl]ox]-9,11,13,13-tetramethyl-12,16-dioxo-1-oxacyclohexadec-4-en-2-yl]- 1H-benz-imidazol-2-yl]-pyrrolidine-1-carboxylate (135).



^{1}H-NMR(400 MHz, CD_3OD): δ(ppm): E-isomer 8.26(s,1H, H-4'), 7.71(d, J=3.2 Hz, 1H, H-7'), 7.19(m, 1H, H-6'), 5.60(m, 1H, H-5), 5.32(m,1H, H-2"), 5.19(m, 1H, H-2), 5.05(dd, ^{3}J=1.92 Hz, ^{3}J=7.96 Hz, IH, H-4), 4.38(dd, ^{3}J=2.0 Hz, ^{3}J=8.0Hz, IH, H-14), 3.85-3.50 (m,2H,H- 5"), 3.24(dd, ^{3}J=1.2 Hz, ^{3}J=6.4Hz, H-15), 3.07(dq, ^{3}J=8.2 Hz, ^{3}J=6.4Hz, IH, H-11), 2.55(m, 2H, H-3"), 2.79(m,2H, H-3) 2.49-2.35(m, 1H, H-7), 2.21-2.11(m, 1H, H-6), 2.05 (m, 2H, H-4"), 1.96-1.88(m, 1H, H-6), 1.57-1.53(m, 3H, H-7, H-8, H-9), 1.28(s, 9 H, 20-H), 1.16(S, 3H, 16-H), 1.1- 1.08(S, 3H, 17-H), 0.94(d, ^{3}J=8.0 Hz, 3H, 19-H), 0.89, 0.87(2S, 2 x 9H, $OSiC(CH_3)_3$),0.08, 0.06, 0.03,-0.33(4s, 4 x 3H, $OSi(CH_3)_3$).

^{13}C-NMR(100 MHz, CD_3OD): $\delta(ppm)$ E-Isomer 217.6(S, C-12), 172.1(S, C-1), 159.2(d, C-2'), 141.5(d, C-5'), 139.1(d, C-5), 122.3(d, C-6'),121.6(t, C-4), 120.3(d, C-7'), 110.5(s, C-4'), 75.9(d, C-14), 70.5(d, C-2), 57.2(t, C-2"), 52.0(t, C-13), 46.8(t, C-5") 44.2(t, C-15) 43.6(d, C-11), 42.4(t, C-3), 32.5(t, C-3"), 31.8(t,

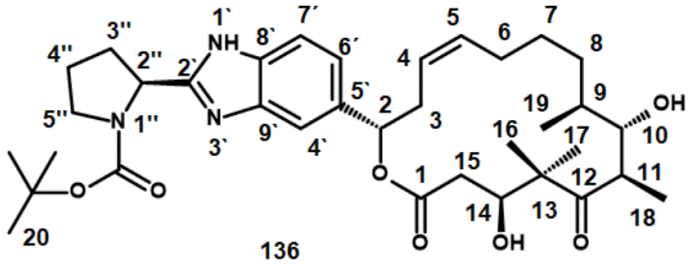
C-6), 30.39(t, C-8), 27.19(t, C-7), 27.72(S, C-20) 26.75, 26.13(q, -OSiC$(CH_3)_3$), 24.93(q, C-17'), 24.8(q, C-17), 22.6(t, C-4"), 18.7(S, OSiC$(CH_3)_3$), 16.13 (q, C-18), 14. -4.12, -4.28, -4.89(q, OSi$(CH_3)_2$).

IR(Film) Z/E Mixture: $\widetilde{\nu}(cm^{-1})$: 2956.15 (S), 2930.83 (S), 2858.03 (S), 2738.59 (w), 2709.65 (w), 2247.49 (m), 1920.05 (w), 1827.29 (w), 1727.97 (S), 1689.2 (S), 1641.90 (m), 1619.65 (m), 1547.57 (w), 1472.96 (S), 1390.58 (S), 1367.00 (S), 1304.14 (S), 1256.55 (S), 1173.89 (S), 1120.63 (S), 1088.45 (S), 988.44 (S), 874.57 (S), 743.10 (S),

MS(EI) **:** m/z(%): 853.3(<1, [M$^-$]), 797.2(100), 740.1(50), 693.1(10), 625.4 (12), 535.4(6), 411.0(5), 327.0(94), 225.0(26), 142.3(3), 109.0(9), 73.0(13)

HRMS(EI): calculated : 854.3235
found : 854.3214

5.8.3 *tert*-Butyl (2*S*)-2-[5-[(2*S*,4*Z*,9*S*,10*S*,11*R*,14*S*)-10,14-dihydroxy- 9,11,13,13-tetramethyl-12,16-dioxo-1-oxacyclohexadec-4-en-2-yl]-1H-benzimidazol-2-yl]-pyrrolidine-1-carboxylate(136).



136

To the solution of **134** and **135** (17 mg, 0.014 mmol) in CH_3CN (1.2 mL) was added a solution made of $(HF)_3.Et_3N$ (0.76 mL, 4.6 mmol) and Et_3N (0.06 mL, 10% v/v) . The mixture was heated in a 45 °C oil bath for 20 hrs, after which it was cooled to room temperature and added to EtOAc (40 mL). The mixture was washed with 5% KH_2PO_4 (aq.) (2 x 30 mL) and the combined aqueous layers extracted with EtOAc (4 x 20 mL). The combined organic extracts were washed with brine (2X), dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (silica gel, EtOAc/pentane 1:1) to afford **136**

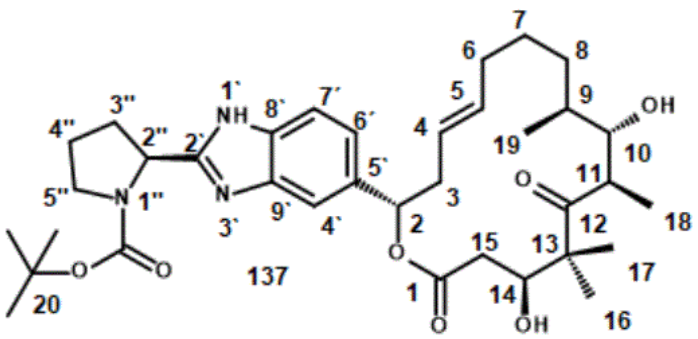
and **137** (8.3 mg, 70%) as yellowish oil. Note: Preparative TLC was used for further purification but unfortunately without success. The product seemed to be unstable to TLC plates.

General Data: $C_{35}H_{51}N_3O_7$, FW:625.80, TLC :R_f=0.12(EtOAc/Pentane 1:3), UV(+), Vanillin: black

^{1}H-NMR(400 MHz, CD_3OD): δ(ppm): Z-isomer 7.73-7.69(m,1H, H-4'), 7.54-7.51(m, 1H, H-7'), 7.41-7.36(m, 1H, H-6'), 5.47-5.32(m, 2H, H-4, H-5), 5.32(m,1H, H-2"), 5.19(m, 1H, H-2), 4.27(dd, ^3J=2.0 Hz, ^3J=8.0Hz, IH, H-14), 3.96(m, 1H, H-2"), 3.55(m, 1H, H-10), 3.05(br s, 1H, OH), 2.7-2.78(m, 1H, H-3), 2.49(dd, ^3J=2.0 Hz, ^3J=8.0Hz, 2H, H-15), 2.55(m, 2H, H-3") 2.35-2.18(m, 2H, 2-H, H-6), 2.01-1.99(m, 2H, H-8), 1.39-1.17(m, 3H, H-8, H-9), 1.34(S, 3H, H-16)1.29(S, 9H, 20-H), 1.09(S, 3H, H-17), 1.00(d, ^3J=4.0 Hz, H-19).

^{13}C-NMR(100 MHz, CD_3OD): δ(ppm) Z-Isomer 220.6(S, C-5), 173.25(S, C-1), 159.2(d, C-2'), 142.1(d, C-5'), 130.2(d, C-12),129.69(t, C-6'),127.87(d, C-13) 126.21(d, C-7'), 120.4(d, C-6') 111.3(s, C-4'), 80.73(q, C-20), 76.2(d, C-2), 75.03(d, C-10), 72.7(t, C-14), 57.2(t, C-2"),53.4(S, C-13), 47.36(t, C-5"), 41.09(d, C-11), 37.81(t, C-7), 34.38(d, C-9), 31.90(t, C-3), 29.76(t, C-8), 29.08(q, C-20), 27.92, 27.21(t, C-10, C-11), 22.96(q, C-17), 15.70(q, C-19).

5.8.4 *tert*-Butyl (2*S*)-2-[5-[(2*S*,4*E*,9*S*,10*S*,11*R*,14*S*)-10,14- dihydroxy-9,11,13,13-tetramethyl-12,16-dioxo-1-oxacyclohexadec-4-en-2-yl]-1H-benzimidazol-2-yl] pyrrolidine-1-carboxylate (137)



^{1}H-NMR(400 MHz, CD_3OD): δ(ppm): E-isomer 7.73-7.69(m,1H, H-4'), 7.54-7.51(m, 1H, H-7'), 7.41-7.36(m, 1H, H-6'), 5.39-5.34(m, 2H, H-4, H-5), 5.32(m,1H, H-2"), 5.19(m, 1H, H-2), 4.16(dd, ^{3}J=2.0 Hz, ^{3}J=8.0Hz, IH, H-14), 3.96(m, 1H, H-2"), 3.42(m, 1H, H-10), 3.05(br s, 1H, OH), 2.69-2.63(m, 1H, H-3), 2.55(m, 2H, H-3"), 2.49(dd, ^{3}J=2.0 Hz, ^{3}J=8.0Hz, 2H, H-15), 2.29-2.05(m, 2H, 2-H, H-6), 2.01-1.99(m, 2H, H-8), 1.39-1.17(m, 3H, H-8, H-9), 1.34(S, 3H, H-16), 1.29(S, 9H, 20-H), 1.07(S, 3H, H-17), 0.94(d, ^{3}J=4.0 Hz, H-19).

^{13}C-NMR(100 MHz, CD_3OD): δ(ppm) E-Isomer 219.6(S, C-5), 173.5(S, C-1), 159.2(d, C-2'), 142.1(d, C-5'), 130.0(d, C-12),129.69(t, C-6'),128.06(d, C-13) 126.21(d, C-7'), 120.4(d, C-6') 111.3(s, C-4'), 80.73(q, C-20), 76.2(d, C-2), 75.03(d, C-10), 72.7(t, C-14), 57.2(t, C-2"),53.4(S, C-13), 47.36(t, C-5"), 41.09(d, C-11), 37.81(t, C-7), 34.38(d, C-9), 31.90(t, C-3), 29.76(t, C-8), 29.08(q, C-20), 27.92, 27.21(t, C-10, C-11), 22.96(q, C-17), 15.78(q, C-19).

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